ASSESSMENT OF THE BIOLOGY, ECOLOGY AND GENETIC STRUCTURE OF THE SEA CUCUMBER CUCUMARIA FRONDOSA FOR MANAGEMENT OF THE FISHERY IN THE NEWFOUNDLAND AND LABRADOR REGION

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ASSESSMENT OF THE BIOLOGY, ECOLOGY AND GENETIC STRUCTURE OF THE SEA CUCUMBER *CUCUMARIA FRONDOSA* FOR MANAGEMENT OF THE FISHERY IN THE NEWFOUNDLAND AND LABRADOR REGION

By

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ABSTRACT

The biological traits of the sea cucumber *Cucumaria frondosa* relevant from both ecological and resource management perspectives were investigated; including distribution, growth and predation on juveniles and adults, and population genetics. Natural densities were mainly dependent on substrate type and location. Spawning occurred from March to May in response to increasing phytoplankton abundance and larvae settled in ca. 5 weeks. Growth of juveniles and adults was slow (indicating ca. 25 years to reach market size) with seasonal patterns correlated with food supply. Juveniles of *S. endeca* fed upon post-metamorphic sea cucumbers, whereas adult feeding rates were modulated by temperature and biased towards small and injured sea cucumbers, suggesting that trawling may exacerbate predation pressure. Population genetic parameters are consistent with persistence in refugia through the last glacial maximum and local genetic patchiness is likely due to variations in recruitment. A precautionary management approach is advisable to ensure the sustainability of the fishery.

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LIST OF ABBREVIATIONS AND SYMBOLS

AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BW	Body wall of the sea cucumber.
CL	Contracted length; length of a sea cucumber that is stimulated to contract measured from mouth to anus.
COI	Cytochrome oxidase I gene region of the mitochondrial DNA.
CPUE	Catch per unit effort: The amount of catch that is taken per unit of fishing gear per unit of fishing time.
CW	Contracted width; width of a sea cucumber that is stimulated to contract measured medially.
$\mathbf{D_{cdf}}$	Cumulative density frequency statistic in SAShA; the average root mean squared difference between the observed and expected cumulative distributions across all geographic distance bins.
DFA	Department of Fisheries and Aquaculture, Government of Newfoundland and Labrador, Canada.
DFO	Department of Fisheries and Oceans Canada.
dNTP	Deoxynucleotide Triphosphate
DW	Dry weight: the weight of a sea cucumber dried in an oven at 60 °C for 5-6 days until constant weight.
EtOH	Ethanol
$\mathbf{F_{ST}}$	F-statistic/fixation index: a measure of the genetic distance between two groups based on genotypic frequencies.
$\mathbf{\Phi_{CT}}$	Φ-statistic corresponding to the correlation between the variance of defined groups compared to all sampled populations.
$\mathbf{\Phi_{SC}}$	Φ-statistic corresponding to the correlation between populations within groups compared to the variance of defined groups.

Φ_{ST}	Φ-statistic corresponding to the correlation between all populations compared to the total variance of all sampled populations.
IW	Immersed weight; the weight of an animal suspended in water measured in grams.
L	Length of a preserved sea cucumber from mouth to anus.
mtDNA	Mitochondrial DNA.
R	Arm radius of a sea star measured from the mouth to the tip of the longest undamaged arm.
PCR	Polymerase chain reaction
SAShA	Statistical analysis of shared alleles; a statistical analysis that compares the spatial arrangement of shared alleles to the expectation under panmixia.
SI	Size index: contracted length multiplied by contracted width multiplied by a scaling factor of 0.1.
SW	Split weight: weight of a sea cucumber cut ventrally from the mouth to a few centimetres before the anus with the internal fluid drained. Measured in grams.
Ti/TV	Transition/transversion ratio: The ratio of the number of transitions to the number of transversions for a pair of sequences.
W	Width of a preserved sea cucumber measured in the middle of the animal.
WW	Wet weight; blotted weight of an animal.
ybp	Years before present.

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CO-AUTHORSHIP STATEMENT

The research described in this thesis was carried out by Justin So, with guidance from Annie Mercier, Jean-Francois Hamel and Sven Uthicke. Justin So was responsible for data collection and analysis. Manuscripts resulting from this thesis were prepared by Justin So, with editing assistance and intellectual input from co-authors as follows:

Authorship for **Chapter 2** is J.J. So, J.-F. Hamel and A. Mercier.

Authorship for **Chapter 3** is J.J. So, S. Uthicke, J.-F. Hamel and A. Mercier

CHAPTER 1: INTRODUCTION

Sea cucumber dish from Stone House Restaurant, St. John's, NL, Canada with the "flower" and body wall.

1.1 Sea cucumber fisheries

The demand for sea cucumbers, or holothuroids, dates to the 16th and 17th centuries in China where they were touted as an invigorating health food. Their name in Chinese; "Hai-shen" translates to "Ginseng of the sea". Today, China remains the main market for sea cucumber products and the global fisheries in Asia and the Indo-Pacific Islands remain active due to the high and ever growing demand (Chen 2004). Currently 90 % of the fisheries target tropical aspidochirotid species (e.g. *Holothuria atra*, *H. scabra, Stichopus horrens*), however more recently a few dendrochirotid species have also been fished, mainly in temperate waters (e.g. *Cucumaria frondosa*, *Athyonidium chilensis*, *Pattalus mollis*; Toral-Granda et al. 2008). The fisheries in Asia and the Pacific region exploit over 80 species of sea cucumbers, resulting in catches of 20 000-40 000 tonnes per year (Toral-Granda et al. 2008).

The main commercial sea cucumber product is "beche-de-mer" made from the boiled and dried body wall of the animal. Fresh or frozen body wall, muscle bands, gonad and oral tentacles are also popular products. Few parts are discarded in processing as nearly 90 % of the animal is edible. Further research into the nutritional value of sea cucumbers indicates that it is low in fat, high in protein and contains many essential amino acids. Recently, dried sea cucumbers in capsule form have been marketed as a health supplement that can be a useful treatment for anemia, arthritis, ulcers and cancer (Chen 2004; Mamelona et al. 2007).

Unfortunately, holothuroids are quite vulnerable to overfishing as demonstrated by the numerous examples of "boom and bust" fisheries, which have brought some sea

cucumber populations close to local extinction (Toral-Granda et al. 2008). Sea cucumber populations in Fiji were quickly depleted within a decade following the re-opening of the fishery in the early 1990's, reaching exports of over 700 tonnes of beche-de-mer product (Kinch et al. 2008). This pattern, where harvesting precedes any kind of management strategy or biological research, is often the norm. Scientific studies are now being conducted in many countries on several species of commercially important holothuroids in an attempt to restore their populations. Sea cucumbers are generally slow moving, have low recruitment rates, and are easy to harvest (Levitan and Petersen 1995; Uthicke and Conand 2005). Combined with the high value of some species, this provides a good recipe for overexploitation and in some areas illegal harvesting (Bruckner et al. 2003). Overfishing can also lead to a prolonged recovery time for the fishery when populations are brought down to very low densities. Furthermore, most holothuroids are broadcast spawners and are subject to Allee effects as they generally depend on close proximity of individuals to ensure acceptable fertilization rates (Uthicke and Conand 2005). Lower than normal densities of sea cucumbers could thus lead to impaired annual recruitment.

1.2 Overview of the biology of *Cucumaria frondosa*

The decline in harvests of traditional fisheries species in Canada has led to a shift in focus to underutilized species. Several exploratory fisheries are being conducted to determine if underutilized species would be commercially sustainable. At the forefront of emerging fisheries in Atlantic Canada is the orange-footed sea cucumber, *Cucumaria*

frondosa. A common holothuroid in North Atlantic and Arctic waters, *C. frondosa* has been well researched due to its wide distribution and potential as a fishery.

Cucumaria frondosa is a dendrochirotid sea cucumber (Echinodermata: Holothuroidea) with ten branched oral tentacles ranging in colour from orange to black. This species has a football shape with a leathery skin ranging in colour from yellowish white to dark brownish-black and is covered with five rows of retractile tube feet (ambulacral podia) (Jordon 1972; Gosner 1978). Three of these bands of tube feet are found ventrally whereas the dorsal rows are often reduced into papillae. Adults of *C. frondosa* have a reduced numbers of spicules (skeletal structures) shaped like rounded plates with regular perforations that range in size from 160 to 350 μm (Levin and Gudimova 2000). The species is sexually dimorphic with a conspicuous tube-shaped (female) or heart-shaped (male) gonopore located under the crown of oral tentacles (Hamel and Mercier 1996a).

Cucumaria frondosa generally inhabits rocky areas where the bottom consists of ledges or boulders and is well distributed from shallow water to depths of more than 300 m (Klugh 1923; Coady 1973; Gosner 1978). Mature individuals have even been collected from depths of 1300 m (J.-F. Hamel, personal observation). Although *C. frondosa* has been found on a variety of substrates such as sand and gravel, it prefers hard rocky substrates (Hamel and Mercier 1996a; Singh et al. 2001). *Cucumaria frondosa* is the most common sea cucumber in the North Atlantic and is found in densities of 5-50 individuals m^{-2} (Hamel and Mercier 1995; Singh et al. 2001) although its distribution is patchy in some areas (Therkildsen and Petersen 2006). Higher densities are observed in areas where currents provide a good source of food (Singh et al. 1998). The species has

an arctic and subarctic distribution along the North Atlantic including the Norwegian, Barents and North Seas. Its southern range extends to the North Shore of Cape Cod in the western Atlantic and to the south of Scandinavia and northern British Isles in the eastern Atlantic (Jordan 1972; Coady 1973; Smirnov 1994). As suspension feeders (Singh et al. 1998; Hamel and Mercier 1998) and prey to several other species (Legault and Himmelman 1993; Francour 1997; Hamel and Mercier 2008), adults of *C. frondosa* form a key component of many cold-temperate ecosystems.

Like other dendrochirotid sea cucumbers, *C. frondosa* captures particles in the water column (Hamel and Mercier 1998). As shown in other species of sea cucumbers, adhesive material is secreted by papillae onto the finely branched tentacles as a means of physiological ensnarement (Fankboner 1978). Particles are ingested when individual tentacles are inserted into the mouth one at a time (Singh et al. 1999). Studies in the St. Lawrence Estuary showed that *C. frondosa* is not a very selective feeder and captures living (planktonic cells, eggs, embryos) and non-living particles, from 25-350 μm in diameter. Intestinal structure, however, seems to be designed primarily for digesting refractory plant material (Hamel and Mercier 1998). *Cucumaria frondosa* shows distinct seasonal feeding patterns dependant on daily food abundance (Jordan 1972; Sutterlin and Waddy 1976; Hamel and Mercier 1998; Singh et al. 1999). Laboratory and field observations indicate that feeding rate is related to food abundance as detected by the oral tentacles, which contain chemosensory and mechanosensory cells (Fankboner 1978; Hamel and Mercier 1998; Singh et al 1999).

The main predators of sea cucumbers include fishes, sea stars and crustaceans, although sea stars are the dominant and best known predators in North Atlantic waters

(Legault and Himmelman 1993; Francour 1997). The predatory sea star *Solaster endeca* shares a similar arctic and subarctic distribution with *C. frondosa* (Gosner 1978). It also has a comparable spawning period (Britt and Petersen 1982; Medeiros-Bergen et al. 1995). *Solaster endeca* has been observed feeding on various holothuroids (Mauzey et al. 1968; Gaymer et al. 2004) and other echinoderms (Bull 1934; Mayo and Mackie 1976); however well developed escape responses of *C. frondosa* to *S. endeca* suggest they have cohabited for a very long time. In the presence of *S. endeca*, *C. frondosa* undergoes strong elongation and contraction motions, making it harder for the sea star to wrap its arms around the sea cucumber (Legault and Himmelman 1993). Unlike *C. frondosa*, the sea star generally exhibits low densities (0.001-0.100 ind m^{-2}) throughout its geographic distribution (Mauzey et al. 1968; Himmelman and Dutil, 1991; Hamel and Mercier, 1996a; Ellis and Rogers 2000; Zolotarev, 2002; Gaymer et al., 2004). Sea cucumbers have also been found to be consumed by wolffish and walruses (Hamel and Mercier 2008) and larval/juvenile sea cucumbers are subject to predation by grazing species such as the green sea urchin *Strongylocentrotus droebachiensis* (Hamel and Mercier 1996a, 2008).

Gametogenesis in *C. frondosa* is initiated in January in correlation with increasing photoperiod. Spawning occurs annually between February and June with variations among regions (Hamel and Mercier 1996b, d, 2008; Singh et al. 2001). High phytoplankton abundance and tidal cycles act as spawning cues (Hamel and Mercier 1995, 1996b, 1999, 2008). Males release spermatozoa first which triggers subsequent release of large buoyant reddish oocytes from females (Hamel and Mercier 1996d, 2008). At depths where there is little light penetration, gametogenesis is triggered through

mucus-mediated chemical communication with conspecifics from populations in the shallower photic zone (Hamel and Mercier 1996b, c, 1999).

Gametes are produced in the gonadal tubules which undergo synchronous or asynchronous development depending on the region or latitude (Hamel and Mercier 1996a). Sea cucumbers with uniform gonadal tubules are associated with areas that have a long and intense feeding period enabling them to develop their gonads and release mature gametes from the entire gonadal tubules in a single year. In regions with lower food abundance or a restricted feeding period, sea cucumbers simultaneously produce two sets of tubules, each requiring two years to develop mature gametes. *Cucumaria frondosa* from these regions are still able to spawn annually by having one set of tubules with mature gametes ready for spawning every year (Hamel and Mercier 1996b).

The buoyant, fertilized sea cucumber eggs occur in high densities in the water column after massive spawning (>95 m^{-3}) and undergo pelagic lecithotrophic development, staying in the water column for ca. 6 weeks, depending on environmental conditions (Hamel and Mercier 1996a, d; Medeiros et al. 1995). Each oocyte is around 0.5-0.8 mm in diameter and estimates of annual fecundity range from 8,000-12,000 mature oocytes (Hamel and Mercier 1996b) to 60,000-150,000 total oocytes per female (Falk-Peterson 1982; Hamel and Mercier 2008). The pentactula larvae settle preferentially on shaded hard substratum including crevices between pebbles and rocks or on shells, kelp and rhodoliths (Medeiros-Bergen et al. 1995; Hamel and Mercier 1996a, 2008; Medeiros-Bergen and Miles 1997). In the St. Lawrence Estuary, growth of *C. frondosa* is slow, with individuals growing from fertilized eggs to a maximum of 102 mm in 40 months (Hamel and Mercier 1996a). Sexual maturity occurs when individuals reach

lengths of 80-102 mm. No studies have followed the growth of the sea cucumber to their maximum size (ca. 350 mm), but it has been estimated to take over 10 years (Hamel and Mercier 1996a). Growth rates seem to be influenced directly by environmental conditions such as temperature and food availability.

1.3 Emerging *Cucumaria frondosa* fishery

The fishery for *C. frondosa* was successfully started in Maine (USA) in the 1980's, and the feasibility of a similar fishery in Canada was assessed soon thereafter (Therkildsen and Petersen 2006; Hamel and Mercier 2008). Main commercial products derived from this sea cucumber include the dried body wall (beche-de-mer), the frozen muscle bands and the flower (aquapharyngeal bulb) (Figure 1-1). The Scotia-Fundy sector, which encompasses Nova Scotia and New Brunswick, has the oldest and largest *C. frondosa* fishery in Canada. Until 2008, the fishery was limited to five exploratory licenses and harvests were over 1,000 tonnes of sea cucumber annually (Therkildsen and Petersen 2006; Hamel and Mercier 2008). Between 2006 and 2008, Newfoundland had up to eight exploratory licenses for the St. Pierre Bank, with combined annual quotas of over 600 tonnes. Sea cucumber bycatch from the scallop fishery were also processed in Québec (Hamel and Mercier 1998) where an official fishery has yet to develop (Hamel and Mercier 2008). The Maine (USA) fishery has recorded annual landings up to 4650 tonnes over the past decade, higher than any other sea cucumber fishery in the United States (Bruckner 2005). There is a small sea cucumber fishery in Iceland; however its future is uncertain due to the low abundance and patchy distribution of populations in that area. No other European country directly fishes sea cucumbers. Based on available data,

Russia has a small unregulated bycatch fishery for sea cucumbers where landings do not exceed 250 tonnes per year (Therkildsen and Petersen 2006; Hamel and Mercier 2008). Already, overfishing of *C. frondosa* populations has been reported in Maine, where effort was concentrated within a few areas (Gudimova et al. 2004; Bruckner 2005; Hamel and Mercier 2008).

Unlike most other sea cucumbers that are hand caught by divers or snorkelers, *C. frondosa* is fished with a modified scallop dredge designed specifically for catching sea cucumbers and minimizing bycatch (Barrett et al. 2007; DFA 2002). The depth of the water where the sea cucumbers are found (ca. 20-100 m), cold temperatures (0-8 °C), poor water transparency and high local biomasses that can reach 50 kg m^{-2}, prompted the development of industrial methods for capture (Hamel and Mercier 2008). The use of towed gear allows harvesters to exploit deeper waters and increase their catch per unit effort (CPUE), however towed gear has inherent negative effects on benthic habitats. The impacts of sea cucumber trawl gear have not been researched specifically, but there are numerous studies on the effects of scallop trawling. Although, the effects on bottom dwelling species depends on how intensively the area was fished (Stokesbury and Harris, 2006), trawls generally damage invertebrates by crushing them and flipping over rocks on top of them (Veale et al. 2000a; Robinson et al. 2001). In addition, the presence of damaged animals attracts predators and scavengers to the area, putting further pressure on the benthic communities (Veale et al. 2000b; Robinson et al. 2001).

1.4 Background information for the present study

Cucumaria frondosa has been described as having a strong preference for hard rocky substrates throughout its life history (Hamel and Mercier 1996a); however, natural populations have been previously observed in areas with soft bottoms (Klugh 1923). In fact, camera tow surveys on the St. Pierre Bank south of insular Newfoundland (Canada) showed that sea cucumbers on sand-dominated areas exhibited unusual rolling and tumbling behaviours and had their tentacles retracted (CSAR 2006). Using laboratory trials, Hamel and Mercier (1996a) found that larvae of *C. frondosa* settled preferentially on rocks or gravel over all other substrates tested (mud, sand). The higher density of sea cucumbers observed on cobbles and rocks than on sandy substrate in nature further supports these results. Differences in habitat could potentially affect the growth and morphology of adults. Rogers-Bennett et al. (1995) observed that the red sea urchin (*Strongylocentrotus franciscanus*) has significantly heavier gonads and shorter spines in shallow waters compared to deeper waters, and suggested shifting harvesting emphasis to sea urchins at deeper levels that have less reproductive potential as a management strategy. Similarly, if sea cucumbers on sand substrates were found to have decreased fitness or reproductive potential, they would be good candidates to harvest, reducing the impact on healthy reproductive populations.

Growth studies are important for determining quotas for a fishery. However the problem associated with measuring sea cucumbers is the plasticity in their physical characteristics. Length, width and wet weight are all dependant on the water content within the sea cucumber. Nonetheless, researchers have devised numerous creative ways

of studying holothuroid growth rates. Growth rates have been established for several species including *C. pseudocurata* (Rutherford 1973), *C. elongata* (Fish 1967), *Isostichopus fuscus* (Herrero-Pérezrul et al. 1999) and *Actinopyga echinites* (Wiedemeyer 1994). In the St. Lawrence Estuary, the growth curve was established for *C. frondosa* (Hamel and Mercier 1996a); however growth was extrapolated from length increments of small individuals (initial size of 35 mm) in the field in areas where phytoplankton levels are greater than around Newfoundland. The St. Lawrence Estuary has high chlorophyll levels of 1.0 mg Chl m^{-3} (Afanasyev et al. 2001) for 11 months of the year compared to only 3 months of the year in Newfoundland. It is therefore important to determine the most accurate way of measuring growth in sea cucumbers and use it to assess if and how Newfoundland sea cucumbers deviate from the growth rate of other, presumably better fed populations.

Predation on holothuroids has rarely been studied even though it can have major impacts on population structure and dynamics. Sea cucumbers are a major part of the diet of some predatory sea stars. In the North East Pacific, *C. lubrica* made up between 58-89 % of the stomach contents of four asteroids, including *Solaster endeca*, *S. stimpsoni*, *Dermasterias imbricate* and *Leptasterias hexactis* (Birkeland et al. 1982). Although sea cucumbers were a major part of their diet, the sea stars were estimated to remove only ca. 3 % of the local population annually (Birkeland et al. 1982). Francour (1997) identified *Crossaster papposus*, *Asterias rubens* and *S. endeca* as sea star predators of *Cucumaria* species; however *C. papposus*, and *A. rubens* are more likely scavengers, feeding on the remains of sea cucumbers rather than active predators (Emerson 1973). Sea stars can have a major influence on population dynamics of commercial species and their impact may

even be exacerbated by fishing activities (MacKenzie and Pikanowski 1999; Zolotarev 2002).

Genetics can be a powerful tool for fisheries management, enabling species identification, and determination of stock structure or origin (Ward 2000). Determination of population genetic structure is especially important for management of broadcast spawning holothuroids due to the high dispersal abilities of their larval stages. If a fishery consists of more than one stock, separate assessment should be made to determine a sustainable catch rate for each. A stock that crosses political or management boundaries would require management at a larger scale (e.g. federal rather than provincial, Ward 2000; Uthicke and Benzie 2001, 2003). *Cucumaria frondosa* has a wide distribution throughout the North Atlantic (Jordan 1972; Gosner 1978). Does this consist of one single stock that interbreeds or separate stocks that do not interact? For instance, do sea cucumber larvae produced in Greenland waters travel south to contribute to the populations in Labrador or Newfoundland? A population structure analysis of putatively separated populations distributed in the North Atlantic is crucial in determining to what extent they interact.

The emerging fisheries policy in Canada provides guidelines to ensure economic viability and sustainable harvesting of new commercial species (DFO 2001). In an effort to exploit underutilized commercial species and conserve natural populations, sound scientific data must be collected to provide the basis for effective fisheries management. According to the Department of Fisheries and Oceans (DFO) Canada, these data are required before any official commercial fishery of *C. frondosa* can begin in eastern Canada. Solid knowledge on the local ecology and biology of this species will assist the

government to ensure sustainable harvesting with minimal impacts on natural populations.

1.5 Objectives of research

Research on *C. frondosa* dates to over a century ago; however the emergence of an Atlantic sea cucumber fishery has prompted more recent studies focusing about management-related questions. Studies in Maine, Québec and New Brunswick have provided details on much of the basic biology of this species including settlement, early growth and spatial distribution (Klugh 1923; Hamel and Mercier 1996a), reproductive biology (Hamel and Mercier 1996a, b; Singh et al. 2001) diet and feeding (Hamel and Mercier 1998, Singh et al. 1998), general ecology (Jordan, 1972) and biochemical content of tissues (David and Macdonald 2002; Mamelona et al 2007; Zhong et al. 2007). However, except for the work of Coady (1973) and preliminary assessments made by Grant (2006) and Grant et al. (2006), very few studies have concentrated on populations from Newfoundland waters which are subject to more oceanic conditions.

In this study, key biological aspects of *C. frondosa* were investigated to provide managers with the information necessary to develop sustainable management strategies. The abundance and distribution of this species relative to habitat type were assessed through field surveys and complementary laboratory analyses were conducted to determine whether substrate had any influence on the biometrics and fitness of individuals. Factors affecting population dynamics were established through examination of several components such as spawning periodicity, growth rates, predation pressure and

gene flow. More precisely, I studied predation pressure under various temperature and light regimes and according to the size of predators in an effort to better assess the impact of predation on local sea cucumber populations in light of the abundance of predatory sea stars. Lastly, sources of recruitment to populations were determined by assessing gene flow among *C. frondosa* populations in the North Atlantic. These data were also used to assess whether groups sampled were part of a single inter-breeding population or several populations.

This thesis first presents the ecology, growth and predation pressure as key factors in the management of *Cucumaria frondosa* in Eastern Canada (Chapter 2). This is followed by a chapter on reconciling genetic parameters with life history traits in *Cucumaria frondosa* (Chapter 3). Finally I provide a summary of the research and highlight the main conclusions that are relevant for management of the emerging fishery (Chapter 4).

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1.7 Figures

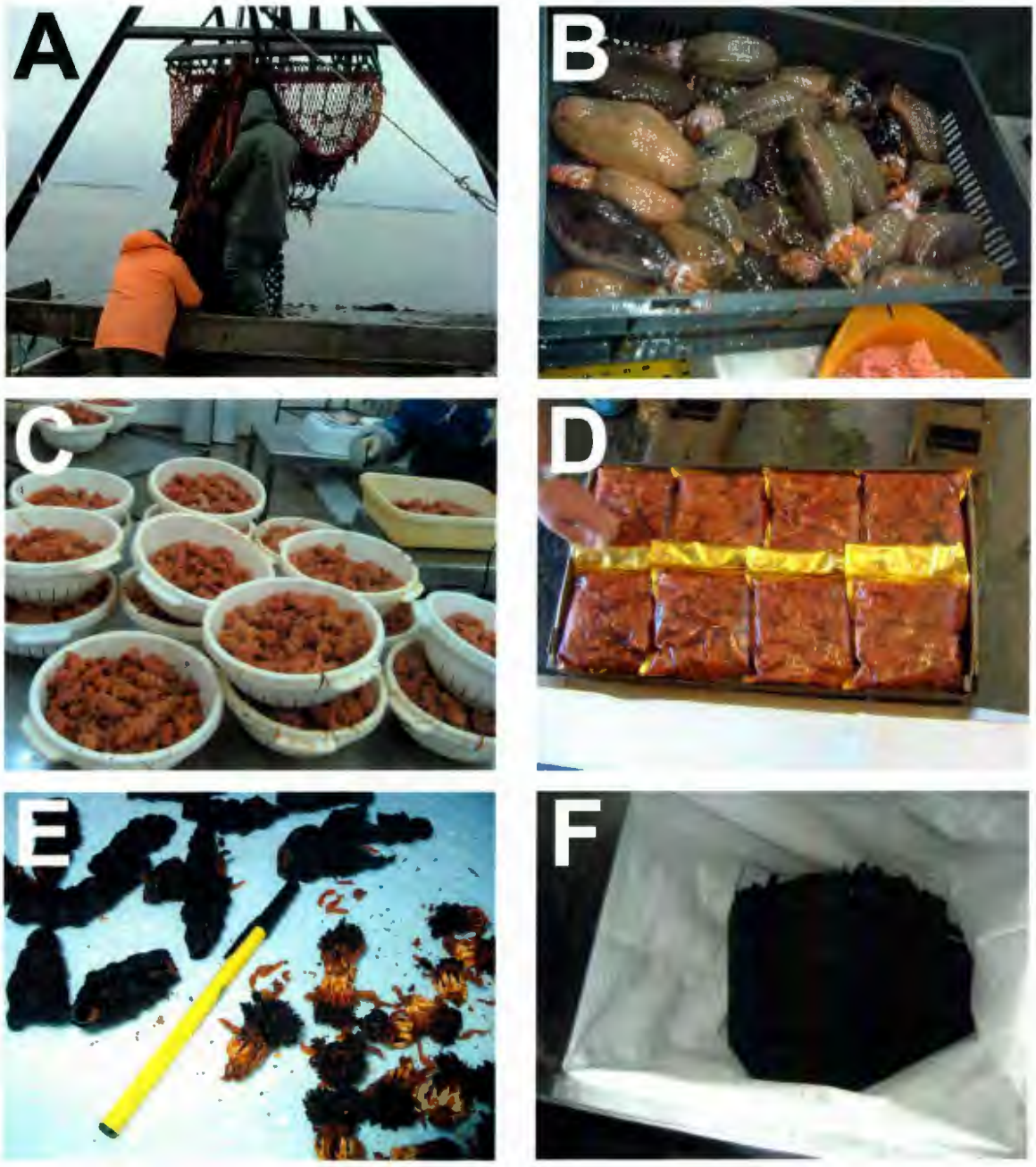

Figure 1-1: Capture and processing of *Cucumaria frondosa*. A) Retrieving the sea cucumber drag, B) whole sea cucumbers in the processing plant, C) gutted circular and longitudinal muscle bands, D) packaged muscle bands ready for shipment, E) dried body wall and aquapharyngeal bulb (flower), F) packaging dried body wall.

CHAPTER 2: ABUNDANCE, GROWTH AND PREDATION PRESSURE IN JUVENILES AND ADULTS OF *CUCUMARIA FRONDOSA* (ECHINODERMATA: HOLOTHUROIDEA): KEY FACTORS IN FISHERY MANAGEMENT

Harvested sea cucumbers on the St. Pierre Bank (courtesy of Lew Barrett)

2.1 Abstract

The sea cucumber *Cucumaria frondosa* is currently the target of an emerging fishery in Atlantic Canada. This study investigated aspects of the biology of *C. frondosa* that are relevant from both ecological and resource management perspectives. Natural abundances (0.03 to 0.29 ind m^{-2}), were dependent on substrate and region. Immersed weight was determined to be the most accurate measure of sea cucumber size and growth. Eight size classes of sea cucumbers kept in tanks under natural conditions of temperature, photoperiod and food supply for 17 months exhibited seasonal growth attuned to spring peaks in phytoplankton abundance. Juveniles obtained from spawning in the laboratory exhibited slow growth, reaching a maximum length of 6 mm after 24 months. Combining growth rates of all size classes indicated that a minimum of 25 years would be required to reach market size (ca. 14 cm contracted length, ca. 6.5 g immersed weight). Under controlled laboratory conditions, adults of *S. endeca* ingested 2-4 sea cucumbers per month with feeding rates modulated by seawater temperature and fasting intervals dependent on prey size. Moreover, juveniles of *S. endeca* preyed on cryptic recruits of *C. frondosa* (<2 y). The maximum density of *S. endeca* in the field was 0.006 ind m^{-2} and its average abundance was 0.001 ind m^{-2} across various substrates. Based on these findings, *S. endeca* has the potential to remove an average of 0.012 ind m^{-2} sea cucumbers annually. Feeding trials also demonstrated that injured sea cucumbers (i.e. damaged by trawling activities) can attract sea stars, which could exacerbate predatory pressure over time. The combination of slow growth rates and high predatory pressure enhanced by fishing activities emphasizes the need for a precautionary approach to managing this emerging fishery.

2.2 Introduction

With a decline in traditional harvests, several exploratory fisheries are being conducted to determine if certain underutilized species would be commercially sustainable. At the forefront of emerging fisheries in Atlantic Canada is the orange-footed sea cucumber, *Cucumaria frondosa* (Echinodermata: Holothuroidea). Relatively new to the market, *C. frondosa* is one of the few dendrochirotid, or suspension-feeding sea cucumbers being fished for human consumption. Existing holothuroid fisheries target about eighty species of aspidochrotid, or deposit-feeding, sea cucumbers (Toral-Granada et al. 2008). Products derived from sea cucumber include the dried body wall (beche-de-mer) and the frozen muscle bands. While sea cucumber fisheries in the Indo-Pacific regions date to several centuries ago, the *C. frondosa* fishery in Canada is currently limited to exploratory licenses granted to harvesters in Nova Scotia, New Brunswick and Newfoundland (Therkildsen and Petersen 2006; Hamel and Mercier 2008a). Sea cucumber bycatch from the scallop fishery is also processed in Québec (Hamel and Mercier 1998), although an official fishery has yet to develop (Hamel and Mercier 2008a). In North America, the *C. frondosa* fishery began in Maine, USA, where annual landings >4.3 million kilograms have been recorded over the past decade (Bruckner 2005; Hamel and Mercier 2008b). Limited and poorly regulated *C. frondosa* fisheries also exist in Iceland and Russia (Gudimova et al. 2004; Therkildsen and Petersen 2006; Hamel and Mercier 2008a).

Developing a sustainable management strategy is a key factor in the emergence of any fishery. Sea cucumber fisheries in particular have a poor history of sustainability, and

species are often overfished due to their slow recruitment rates, ease of catch and high commercial value (Uthicke and Conand 2005; Toral-Granda et al. 2008). In addition, sea cucumbers are known to be vulnerable to Allee effects, where decreasing densities of sexually mature adults cause a disproportionate decline in the number of juveniles produced (Stephens and Sutherland 1999). In most commercial broadcast-spawning species, reduced adult densities that increase the distances between individuals have been shown to reduce fertilization rates and subsequent recruitment (Levitan and Petersen 1995). For instance, low annual catches of 5 % of virgin biomasses of *Holothuria nobilis* on the Great Barrier Reef resulted in severely reduced stocks (Uthicke 2004). Other sea cucumber species that have not seemed to recover from overexploitation include *Isostichopus fuscus* in the Galapagos Islands (Ecuador) and *Holothuria scabra* in Malaysia (Uthicke and Conand 2005; Toral-Granda et al. 2008).

In a global review of the status and management of sea cucumber species that are already harvested or have a commercial potential (Toral-Granda et al. 2008), the case of *Cucumaria frondosa* stands out. Unlike most tropical sea cucumbers that are hand caught by divers or snorkelers, *C. frondosa* has prompted the development of industrial methods for capture (Hamel and Mercier 2008b). Harvesters use a modified scallop trawl designed specifically for catching sea cucumbers and minimizing bycatch (DFA 2002a; Barrett et al. 2007). This method emerged because of the depth of the water where the sea cucumbers are found (ca. 20-100 m), the cold seawater temperatures (0-8 °C), poor water transparency and the high local biomasses of *C. frondosa*, which are known to reach 50 kg m^{-2} in certain areas. While use of towed gear allows harvesters to exploit deeper waters and increase their catch per unit effort (CPUE), it has inherent negative effects on

bottom habitats (Jones 1992). The impacts of the sea cucumber trawl gear have not been specifically researched, but there are a multitude of studies on the adverse effects of scallop trawling on benthic communities (Robinson et al. 2001; Veale et al. 2000a), which largely depend on how intensively the area is being fished (Stokesbury and Harris 2006). Towed gear may inadvertently cause increases in predatory sea stars as it may damage benthic invertebrates, increasing the availability of food (Zolotarev 2002).

Although research on *C. frondosa* began over a century ago, the advent of a northwest Atlantic sea cucumber fishery has prompted new interest in fisheries-related questions. Previous studies have detailed much of the basic biology of this species, including localized spatial distributions, larval development and settlement, juvenile growth and ecology (Hamel and Mercier 1996a), reproductive biology (Coady 1973; Hamel and Mercier 1995, 1996a, b, c; Singh et al. 2001), diet and feeding (Hamel and Mercier 1998; Singh et al. 1998), general ecology (Klugh 1923; Jordan 1972) and biochemical content of various tissues (David and MacDonald 2002; Mamelona et al. 2007; Zhong et al. 2007). However, these studies were mainly conducted on populations from nearshore and estuarine environments of Québec and the Maritimes, whereas data on sea cucumbers in Newfoundland waters are restricted to a preliminary investigation of the reproductive cycle (Coady 1973) and stock assessments (Grant 2006; Grant et al. 2006). Furthermore, the growth of adults and the dynamics of predation pressure have never been investigated in detail anywhere.

Cucumaria frondosa is generally described as having a strong preference for hard substrate throughout its life history (Hamel and Mercier 1996a). The higher density of sea cucumbers observed in the field on cobbles and rocks than on sand further supports these

results. Nevertheless, natural populations have been observed on soft bottoms (Klugh 1923) and recent camera tow surveys on the St. Pierre Bank south of Newfoundland (Canada) showed that *C. frondosa* could occur on predominantly sandy substrate (CSAR 2006). However, most specimens had retracted tentacles and were displaying unusual rolling and tumbling behaviours, raising the question of the status of these populations and whether or not they should be a prime target for a future fishery.

The rate at which fished populations can be replenished largely depends on local recruitment and subsequent growth of new recruits to commercial size. Because of their plastic shape, the variable amount of retained water in their ambulacral system and the stiffness of their muscles bands, sea cucumbers are inherently difficult to measure and weigh (Conand 1979; Pitt 2004). This remains one of the major obstacles in the development of reliable biomass estimates needed to establish harvest quotas. It also complicates assessments of growth and size-at-age. Previous growth studies have used many different methods of measurement, including wet weight, dry weight, size indices based on length and width, gutted weight and variations on these (Cameron and Fankboner 1989; Pitt 2004; Grant 2006). There are merits to these measurements; however they are still largely influenced by the sea cucumber's variable shape and size. The drawback of more reliable measurements such as gutted and dry weight is that they are invasive and require sacrificing the animal, making them unsuitable for repeated growth measurements. Immersed weight, which weighs the specimen in a basket suspended in seawater, is more suited for growth studies of fluid-filled animals. It has been used previously to measure *C. frondosa* size in Russia (Gudimova et al. 2004) and

has proven to be accurate in other marine invertebrates (Muscatine 1961; Palmer 1983; Spirlet et al. 2000, Grosjean 2001).

Predation can have major impacts on fished populations and management plans, especially if harvesting exacerbates it (Steneck 1998; MacKenzie and Pikanowski 1999). The principal predators of sea cucumbers include various species of fishes, sea stars and crustaceans, though sea stars are the dominant predator in North Atlantic waters (Legault and Himmelman 1993; Francour 1997). The main known predator of *C. frondosa* is the sea star *Solaster endeca,* which shares its arctic and subarctic distribution (Gosner 1978) and exhibits an overlapping spawning period (Britt and Petersen 1982; Medeiros-Bergen et al. 1995). This sea star has been observed to successfully feed on various holothuroids (Mauzey et al. 1968; Gaymer et al. 2004) and other echinoderms (Bull 1934; Mayo and Mackie 1976). The well developed escape response of *C. frondosa* exposed to *S. endeca* (Legault and Himmelman 1993) suggests that they typically live in sympatry. Unlike *C. frondosa* and a number of other sea star species, *S. endeca* is known to occur at low densities (0.001-0.100 ind m^{-2}) (Mauzey et al. 1968; Himmelman and Dutil 1991; Hamel and Mercier 1996a; Ellis and Rogers 2000; Zolotarev 2002; Gaymer et al. 2004). Nevertheless, much higher densities of these sea stars have been measured in extensively fished areas of the Grand Banks (J.-F. Hamel, personal observation) where large biomasses of *C. frondosa* were also noted (CSAR 2006). It is therefore essential to estimate the rate of predation by *S. endeca*. Moreover, with the exception of the well studied prey-predator interactions in adults, few studies in marine invertebrates have looked at the impact of predatory pressure on the recruitment of post-metamorphic sea cucumber juveniles.

In this study, key biological aspects of *C. frondosa* were examined which are not only significant from an ecological viewpoint but also provide managers with the tools to develop appropriate management strategies. The abundance and distribution of this species were assessed through field surveys conducted by the Department of Fisheries and Oceans (DFO) on the St. Pierre Bank, Newfoundland and Labrador (Canada). Laboratory analyses were conducted to determine if specimens occurring on suspected biologically non-optimal substrate would exhibit distinct biometric characteristics. Factors impacting population dynamics were established through examination of spawning periodicity and growth rates of various life stages, from larva to adult. Lastly predation rates by *S. endeca* and prey size preferences under various temperature and light regimes were measured and correlated with the abundance of this sea star in the field, to assess its potential influence on local sea cucumber populations. According to DFO, these data are required to determine the potential for a commercial fishery of *C. frondosa* in eastern Canada under the New Emerging Fisheries guidelines (DFO 2001). Solid knowledge on the local ecology and biology of this species will assist the government to ensure sustainable harvesting with minimal impacts on natural populations.

2.3 Materials and Methods

Field studies

Natural abundance in the field

Harvesters, under the direction of DFO, used sea cucumber drags (Barrett et al. 2007) to perform survey tows across inshore and offshore regions of the St. Pierre Bank (Newfoundland and Labrador, Canada; 45°N, 56°W) in 2007 to determine relative abundances of *C. frondosa* and the sea star *S. endeca* (n=214 tows). The 1.8 m wide drags were fitted with 10 cm mesh size nets and were towed at ca. 2.5 knots for a distance of ca. 0.9 km, sampling an area of ca. 1650 m^2 for each tow. Total catches of each species was recorded and substrate type was noted through use of bottom charts, and substrate collected in the nets. Harvesters placed large catches of *C. frondosa* (>40 ind) in totes and logged the numbers of totes filled per tow. Each tote carried ca. 90 sea cucumbers based on number of individuals per tote measured in previous years (E. Hynick personal communication). As such numbers of sea cucumbers and subsequent densities were estimated.

Substrate related condition

In summer 2005, camera observations and sample collection were carried out on the St. Pierre Bank to determine habitat utilization of known populations of sea cucumbers. Video footage was taken of the sea floor using a camera mounted on a benthic sled as described in CSAR (2006). The underwater camera (Simrad OE 1367) was positioned at a downward angle on the front of the sled in the direction of the tow to yield an oblique view of the sea floor. Digital video was stored onboard the sled using a

DeepCam autonomous recording unit and was downloaded and viewed after each tow (CSAR 2006). The sled was towed at a speed of ca. 3.7 km h^{-1} with on bottom durations of 0.09-0.62 h (CSAR 2006).

Video footage was analyzed to identify substrate from five sets of transects (Figure 2-1). Areas were classified into hard, soft or mixed substrates according to the dominant substrate identified (see below). Five sample sets of sea cucumbers (Table 2-1) were collected by trawl from the three representative substrates and preserved in 100 % ethanol (EtOH) for further analysis in the laboratory. A single sample was taken from rock substrate (n=17 in sample site R1), two samples from gravel (n=21 in sample G1; n=15 in sample G2) and two samples from sand (n=23 in sample S1; n=28 in sample S2).

Substrate types

Throughout the study, substrates were classified as hard or soft (Table 2-2). Hard substrates refer to both rocky and gravelly substrates and a mixture of the two. Rocky substrate consists of irregular bedrock bottoms with outcrops and boulders. Gravelly substrates are relatively flat and covered in small rocks (2-5 cm in diameter), shells, and tests of other invertebrates. Hard mixed substrate refers to a combination of rocky and gravelly substrate types with irregular rocky bottoms (outcrops and boulders) covered in small rocks (2-5 cm in diameter) and shells. Soft substrate refers to flat sandy bottoms with scattered rocks (10-15 cm in diameter).

Laboratory studies

Collection and maintenance

Sea cucumbers (*C. frondosa*) and sea stars (*A. rubens* and *S. endeca*) were collected by SCUBA divers at ca. 10-20 m in Admiral's Cove (47°05'N, 52°54'W) and by trawl at ca. 50 m in the St. Pierre Bank, Newfoundland (45°43'N, 56°08'W) between September 2005 and February 2008. Small specimens (contracted length <1.5 cm) were collected in September 2006 as bycatch in whelk pot by fishermen on the St. Pierre Bank. All specimens were brought back to the laboratory within 5-6 h of collection and transferred to 1 m^3 holding tanks provided with unfiltered running seawater at ambient temperature (-1 to 15 °C over the annual cycle). Specimens were subjected to natural photoperiod and a maximum light intensity of 43 $\mu mol\ s^{-1}\ m^{-2}$.

Measurement methods and accuracy

To identify the best method of determining sea cucumber size, we compared several methods to measure and weigh sea cucumbers. The dry weights of 164 sea cucumbers were measured against the contracted length, width, size index, total wet weight and total animal immersed weight to determine their accuracy. For all measurements sea cucumbers were handled in seawater until contracted and were measured for contracted length (CL, from mouth to anus) and contracted width (CW, at the middle of the sea cucumber), total animal wet weight (WW), and immersed weight (IW, by placing the specimen in a basket attached to a scale and suspended in seawater). The size index (SI) was calculated as follows: contracted length multiplied by contracted width multiplied by a scaling factor of 0.1 (Jordan 1972; Cameron and Fankboner 1989; Grant 2006). Dry weight (DW) was obtained by piercing the sea cucumbers' body wall

with a scalpel to release coelomic fluid. The specimen was then placed in an oven at 60 °C for 5-6 days until constant weight was obtained. Sea cucumber measurements were plotted against dry weights and a correlation coefficient of determination was calculated for each comparison.

Sea stars were measured for total wet weight (WW) and maximum radius (R) was recorded by measuring the distance between the mouth and the tip of the longest ray.

Analysis of substrate related condition

To assess body size in relation to substrate, sea cucumbers preserved in EtOH were measured for length (L, from mouth to anus), width (W, at the middle of the animal) and wet weight (WW). Individuals were cut ventrally from the mouth to a few centimetres before the anus and the internal fluid was drained to obtain split weight (SW). The organs (body wall (BW), intestine, gonad, respiratory tree and aquapharyngeal bulb) were then removed and weighed. The thickness of the body wall was measured ventro-medially with a caliper, excluding the circular and longitudinal muscle bands. Gonad and body wall indices were determined by dividing the weight or thickness of the tissue by the weight of the body wall. Substrate groups were compared for each of the measurements recorded.

Growth studies

Temperature was continuously monitored in the tanks using a temperature-light logger (HOBO Pendant UA-002-64), and chlorophyll fluorescence was obtained from DFO from hydrographic station 27 (47°32'N, 52°35'W) located in the inshore branch of the Labrador Current, 12 km south-east of the Ocean Sciences Centre, Logy Bay

(Newfoundland). Data collected from the station are representative of ocean conditions occurring over the Newfoundland shelf (Petrie et al. 1988) and correspond to the biological and physical characteristics of the water supplied to our laboratory (Gotceitas et al 1999).

Growth of laboratory reared juveniles (size Class 1). Adult sea cucumbers exposed to natural environmental conditions in a 600 L tank spawned in March 2006 and the resulting offspring were used to measure development and growth rate. Analogous spawning periods (March-April) were also observed in 2005, 2007 and 2008. Upon spawning, the floating eggs were checked for an elevated fertilization membrane (confirming fertilization) and transferred to a 20 L container with holes covered by mesh within a separate tank provided with running, unfiltered seawater (ca. 30 L h^{-1}). Rocks were provided to allow proper settlement habitat as described by Hamel and Mercier (1996a). Individuals from spawning were classified as size Class 1 (Table 2-3). The embryos/larvae were photographed and measured regularly to record developing stages and kinetics. After settlement the juveniles were monitored once a month. Photographs were taken with a stereomicroscope (Nikon SMZ1500) coupled to a Nikon DXM1200F digital camera. As Hamel and Mercier (1996a) demonstrated that the diet of *C. frondosa* is mainly composed of phytoplankton, unfiltered seawater was supplemented (3.8×10^5 cells ml^{-1}) to ensure a sufficient algal abundance with regular addition of live green algae (*Tetraselmis suecica* and *Isochrysis sp.* Tahitian strain), brown algae (*Nannochloropsis oculata*) and rotifers (*Branchionus plicatilis*) using a peristaltic pump (ca. 0.5 L h^{-1}). After five months, juveniles were transferred to a 26 L tank with rock substrate to give them

more space to grow. Algae and rotifers continued to be added to the tank to promote growth. Juvenile sea cucumbers (n=10-30) were monitored and measured for 25 months.

Growth of juveniles and adults (size Classes 2-8). Because the slow early growth rates already described for *C. frondosa* by Hamel and Mercier (1996a) make it impossible to monitor the entire growth from larvae to commercial size within a reasonable time frame, growth rates measured simultaneously in various individual size classes were combined. Individuals collected from the field were divided into seven groups based on the contracted length using four replicates where sample numbers permitted (Table 2-3). Sea cucumbers from size Classes 2-7 were placed in 26 L tanks under natural photoperiod and light intensity (43 $\mu mol\ s^{-1}\ m^{-2}$) and provided with running unfiltered seawater. Class 8 (largest) individuals were placed in a divided 350 L tank under similar conditions. Stocking biomass did not exceed 50 $g\ L^{-1}$ and densities ranged from 5 to 11 individuals per tank. Temperature in the tanks ranged from 0.5-15 °C following the ambient annual cycle above the seasonal thermocline which sea cucumbers are exposed to in shallow waters (<20 m). For each size class, individuals from four replicate enclosures were measured for contracted length and width, total wet weight and immersed weight either monthly, bi-monthly, after 12 months or after 17 months to control for sampling stress bias on growth.

Growth of food-supplemented juveniles and adults (Classes F4-F6). Complementary studies were conducted as previously described but with algae supplements to determine if increased food supply would affect the growth of *C. frondosa*. Live green algae (*T. suecica* and *Isochrysis* sp. Tahitian strain) and brown algae (*N. oculata*) were continuously added to the tanks (3.8×10^5 cells ml^{-1}) using a

peristaltic pump (ca. 0.2 L h^{-1}). Sea cucumbers corresponding to size Classes 4-6 were used with two replicates of each class. The first replicate was sampled bi-monthly and the second replicate was sampled at the beginning and end of the 17-month experiment.

Predation studies

Early juvenile interactions. As juvenile sea cucumbers dwell under rock surfaces and presumably remain inaccessible to adults of their main predator, the sea star *S. endeca*, we wanted to assess whether juvenile sea stars (1-2 year old) that can access refuge habitats could be a threat to recently settled *C. frondosa*. Adult *S. endeca* exposed to natural conditions in a 250 L tank spawned in April 2006 and their development, settlement and juvenile growth was followed as already described for *C. frondosa*. The first goal was to examine the attraction of juvenile sea star predators toward juvenile sea cucumbers and the second goal was to assess whether the latter had already developed the escape response observed in adult specimens, as described by Legault and Himmelman (1983). One year old *C. frondosa* juveniles (ca. 1.5-2 mm long) reared under laboratory conditions were transferred to a Petri dish filled with seawater and allowed to acclimate for 10-15 minutes. Juveniles were observed for control behaviour prior to adding one or two specimen(s) of *S. endeca* juveniles (ca. 3-3.5 mm in diameter; also reared in the laboratory). Juvenile sea cucumbers were never used more than once and the experiment was considered over when *S. endeca* grabbed the sea cucumber and started evaginating its stomach or if the escape response of the sea cucumber successfully deterred the predator. The experiment was conducted for <15 minutes, was replicated 32 times, and was considered null if no change in behaviour was observed during that interval. Interactions

were observed with a Nikon SMZ1500 stereomicroscope and pictures were taken using a Nikon digital camera DXM1200F.

Feeding behaviour of sea stars. In all prey-predator studies described hereafter, sea stars were considered to be "stationary" when remaining immobile, their arms firmly attached to the substrate and were considered to be "searching" when they moved towards the side of the tank extending their arms and apical tube feet in search of a prey prior to moving towards it. Feeding behaviour encompasses both attacking and eating the prey. Sea stars were considered to be "attacking" when actively engaging and attempting to wrap their arms around the prey and to be "eating" when they were completely wrapped around a sea cucumber and inserting it into their mouth.

Prey size selection and feeding cycle of adult S. endeca. Feeding behaviours of *S. endeca* were monitored from June to August 2007 to determine prey size selection. Three specimens each of medium (R: 11.5 ± 0.1 cm; WW: 382.9 ± 40.7 g) and large (R: 14.5 ± 0.5 cm; WW: 621.5 ± 26.0 g) *S. endeca* were each placed in 57 L tanks provided with running seawater (60 L h^{-1}) at ca. 4-14 °C (ambient seawater temperature varied naturally). A light intensity of ca. 43 μmol s^{-1} m^{-2} was used under naturally fluctuating photoperiod. Prior to the experiments, sea stars were starved for a minimum period of two weeks to standardize their status and stimulate predation as described for other species by Rochette et al. (1994). A small (CL: 6.4 ± 1.8 cm; IW: 0.6 ± 0.3 g) and large (CL: 14.2 ± 1.8 cm; IW: 5.8 ± 1.1 g) sea cucumber was placed in each of the experimental tanks. Observations were noted daily and sea star behaviour was recorded as described above. Upon consumption, sea cucumbers were replaced immediately with a similar sized sea cucumber until the end of the experiment. Whenever a partially eaten

sea cucumber was found it was left in the tank for no more than 48 h before removal and replacement. Within the same experiment, the feeding cycle of *S. endeca* was determined by noting the length of time spent eating each sea cucumber (feeding bout) and fasting intervals between feedings for each sea star individually.

Effects of temperature on feeding of S. endeca. Due to observed responses of sea stars to temperature in the feeding cycle described above, we examined variations in the predation pressure exerted by *S. endeca* on *C. frondosa* in response to the annual cycle of temperature. The feeding response of medium-sized (R: 10.5 ± 1.5 cm, WW: 298 ± 103 g) sea stars was tested at low (6.2 ± 0.3 °C; n=12) and high (14.7 ± 0.4 °C; n=9) seawater temperatures, to which *S. endeca* are exposed in shallow waters (<20 m). The experiment was also repeated with large-sized predators (R: 14.6 ± 1.5 cm, WW: 713 ± 221 g) using 15 and 13 replicate animals for low and high temperatures, respectively. Sea stars and sea cucumbers were first acclimated to the test temperature for 24 h in separate tanks, and *S. endeca* were starved for a minimum of two weeks as for previous predator experiments. A sea cucumber was introduced first and then a sea star 30 minutes later into a 57 L tank with a minimum flow through of sea water (1 L min^{-1}; to allow for control of temperature) and the usual light intensity and photoperiod. Specimens were monitored for up to 6 h during the day and their behaviour was recorded every 30 minutes using the feeding terminology previously described. Experiments were ended as soon as *S. endeca* was observed to attack a sea cucumber. Experiments (n=3) were repeated at night in total darkness with medium-sized *S. endeca* to determine if there was any circadian rhythm in feeding behaviour. Observations were made with a red light. Starved medium-sized sea

stars were used as they were more likely to give a consistent result as they have shorter feeding cessation periods compared to larger sea stars.

Response of S. endeca *to damaged and intact sea cucumbers.* It was hypothesized that sea cucumbers damaged by trawls could attract more predators in a given site and therefore increase the predatory pressure on the population in fished areas. To test this, a pair-wise experimental design was used: a single *S. endeca* along with two sea cucumbers were released haphazardly into an experimental tank to determine if the predator would exhibit any preference for damaged sea cucumbers. The conditions in the tank were as previously described for the feeding cycle study and temperature ranged from 0-2 °C. Twenty *S. endeca* of varying size were tested independently (R: 10-18 cm; WW: 298-1084 g) and offered a choice between an intact and a damaged sea cucumber. The 'damaged' specimen had a 4 cm long cut through the ventral side of the body wall exposing internal organs (i.e. in an effort to mimic severe damage by gear). Each trial ran for 48 h and the reaction of the predator (stationary, attacking, or eating; as described previously), its prey preference (i.e. movement towards damaged or intact sea cucumbers) and the swiftness of the reaction were noted.

Complementary experiments using a single *A. rubens* placed in a tank with a damaged and intact sea cucumber were conducted and interactions observed over 48 hours (n=13 sea stars) at ambient temperatures of 0-2 °C.

Statistical analyses

Statistical analyses were conducted through the statistical package SPSS ver 16.0.1 (SPSS Inc 2007). One way analysis of variance (ANOVA) and Bonferroni

adjusted multiple comparison, were conducted to determine if there were any significant changes in size and differences between groups subjected to different sampling regimes in the growth experiment. Three-way ANOVA was used to determine any significant differences in densities or size measurements of sea cucumbers from various substrates and two-way ANOVA was used to determine effects of predator size and temperature on feeding activity. Data are presented as mean ± standard deviation, where applicable.

2.4 Results

Natural abundance

The majority (69 %, n=149) of all tows across a variety of substrates contained catches of *C. frondosa* (Figure 2-2). *Solaster endeca* occurred in 23 % (n=51) of all tows and were mainly caught in areas with high sea cucumber density. Less than 1 % (n=2) of tows contained *S. endeca* without *C. frondosa*. Three way ANOVA revealed a main effect of substrate type ($F_{(3,94)}$=13.25; p<0.001) and inshore or offshore region ($F_{(1,94)}$=10.855; p=0.001) on *C. frondosa* density. Presence of its predator *S. endeca* ($F_{(1,94)}$=3.765; p=0.055) only had localized effects on *C. frondosa* densities (Figure 2-3). There was a significant interaction between region, presence of predator and substrate type on densities ($F_{(2,94)}$=6.863; p=0.002). Tests of simple effects indicated that sea cucumber densities in areas offshore, only changed with substrate type ($F_{(3,94)}$=8.555; p<0.0001) but not presence of predator (Figure 2-3; $F_{(3,94)}$=0.0007; p=4.322). Inshore areas had significant simple effects of substrate ($F_{(1,94)}$=12.666; p<0.001) and predator presence on sea cucumber densities ($F_{(3,94)}$=9.000; p<0.001). Further tests of significant interaction

between presence of predator and substrate type ($F_{(2,94)}$=4.111; p=0.018) inshore, revealed variations in density across substrates depending on presence ($F_{(2,94)}$=12.555; p<0.0001) or absence ($F_{(3,94)}$=1.666; p=0.218) of *S. endeca*.

Interestingly, relatively high densities of *S. endeca* (0.02 ind m^{-2}) were also found on hard rocky substrate where no *C. frondosa* were caught (n=2, data not shown). The best catch of *S. endeca* in one tow was >300 sea stars yielding an estimated density of 0.05 ind m^{-2}. No significant main effects were found with substrate type ($F_{(3,32)}$=0.510; p=0.679) or region ($F_{(1,32)}$=1.300; p=0.263) on *S. endeca* densities, nor any significant interaction between the two factors. Individuals of *S. endeca* ranged from 41-1136 g (WW) with an average of 248 ± 199 g across the St. Pierre Bank (data not shown). Size structure distributions of *S. endeca* on the St. Pierre Bank and in the Barents Sea (Zolotarev 2002) are shown in Figure 2-4. The majority of sea stars in both areas have an arm radius of 7-11 cm, corresponding to medium-sized sea stars used in this study.

Substrate related characteristics

Morphometric analysis indicated important variability in sea cucumber size among substrate types sampled (Table 2-1). Sea cucumbers from hard rocky substrate were the heaviest (WW: 369 ± 139 g in R1) followed by mixed rocky-gravely substrate (212 ± 67 g in G1; 241 ± 47 g in G2) and soft sandy substrate (197 ± 64 g in S1; 143 ± 47 g in S2). Comparison of individuals from each group indicated no statistical differences in gonad index between females (Figure 2-5) ($F_{(4,36)}$=0.621, p=0.651). Male sea cucumbers from one sandy substrate (S1) had a higher gonad index (42 ± 8 %) compared to all other specimens, although statistical differences occurred with specimens

from the other sandy substrate (S2) and gravely substrate (G1) (Figure 2-5) ($F_{(4,58)}$=4.567, p=0.003). The body wall thickness index was significantly higher in males from sandy substrate (S2) than in males from rocky (R1) and mixed rocky-gravely substrate (G1 and G2) (Figure 2-5) ($F_{(4,58)}$=7.574, p=0.0001). Females from sandy substrate (S2) had similarly higher body wall thickness index compared to the other substrates (R1 and G2) (Figure 2-5) ($F_{(4,36)}$=5.647, p=0.001). Intestine, respiratory tree, and aquapharyngeal bulb weights between individual groups were not significantly different in either males or females (data not shown).

Growth of larvae and laboratory-reared juveniles (size Class 1)

Several spawning events occurred between March and May 2006 during the early morning and afternoon. Spawning females were positioned in a "U" shape with their ventral posterior end attached to a hard surface. The oral end remained unattached with their oral tentacles waving, while the gonopore released a continuous string of buoyant oocytes (ca. 750 μm) that quickly dispersed in the water column. Male spawning occurred earlier and behaviour was similar to that of females. The dispersion of spermatozoa was also aided by the waving of tentacles.

The development of *C. frondosa* from fertilization is detailed in Table 2-4. After 10 days, pentactula larvae had oral tentacles and after 15 days they developed the first two ambulacral podia (Figure 2-6). Settlement began ca. 4.9 weeks after fertilization. By three months, the juveniles possessed clearly visible ossicles along the body wall (Figure 2-7). The juveniles reared in the laboratory from fertilization (size Class 1) reached a maximum length of 5.6 mm after 24 months; the average length was ca. 3.9 mm (Figure

2-8). Juvenile lengths were quite variable over the entire study with the smallest and largest juveniles at the end of the study varying between 2.5 and 5.6 mm. Larger individuals were found on the undersides of rocks, whereas smaller juveniles were generally in more exposed areas. The average monthly growth rate over the 24 months was 0.16 ± 0.06 mm.

Accuracy of measurements on adults

All measurements including length, width, wet weight, immersed weight and size index showed a positive relationship with whole animal dry weight (Figure 2-9). The total animal immersed weight had the strongest relationship (r^2=0.97) and contracted length the weakest (r^2=0.48). Size index had a greater correlation coefficient with dry weight compared to either of its constituent factors (r^2=0.69). As the immersed weight was by far the most precise method to estimate the size of *C. frondosa*, it was used subsequently to measure the growth rate.

Juvenile and adult growth (size Classes 2-8)

Cucumaria frondosa showed seasonal pulses of growth with an overall null growth by the end of the 17-month study (Figure 2-10). Decreases in immersed weight occurred in all size classes from January to May when the temperature fluctuated from -1 to 0 °C but increases occurred throughout June and July when temperature increased from 2 to 4 °C soon after a major increase in phytoplankton level. Due to the seasonal trend of the observed growth, comparisons were made between sizes over a single year (December-December). After 12 months the average immersed weight did not

significantly vary in most size classes (Class 2: $F_{(6,34)}$=1.888, p=0.112; Class 3: $F_{(6,51)}$=0.401, p=0.870; Class 5: $F_{(11,47)}$=1.383, p=0.213; Class 6: $F_{(11,44)}$=4.144, p=0.0001; Class 7: $F_{(11,42)}$=2.475, p=0.017), although decreases in weight were significant in Classes 4 ($F_{(11,104)}$=2.842, p=0.003) and 8 ($F_{(11,93)}$=3.065, p=0.002) (Figure 2-10).

Third and fourth replicates of size Classes 2 to 8 which were measured only at the middle and end of the study were of similar size to replicates measured more often within each size class. One way ANOVAs and multiple comparisons revealed significant differences in replicates of Class 3 ($F_{(1,10)}$=18.42, p=0.002) (Table 2-5), but not in any other class (Table 2-5). Minimal mortalities (<10 %) occurred throughout the study.

Food-supplemented individuals showed visually similar growth curves with increases in immersed weight soon after the spring phytoplankton bloom, although over 12 months the average immersed weight of sea cucumbers did not vary in two classes (Class F4: $F_{(6,22)}$=1.257; p=0.317; Class F6: $F_{(5,20)}$=2.622; p=0.056) and only in May in Class F5 ($F_{(6,28)}$=7.056; p=0.0001). Comparing sea cucumbers with and without algae supplement showed no differences in initial ($F_{(1,14)}$=0.002, p=0.966) or final ($F_{(1,14)}$=1.346, p=0.265) weights for Classes F4 and 4. Significantly higher ($F_{(1,8)}$=5.586, p=0.046) final immersed weights were observed in Class F5 (0.7 ± 0.1 g) compared to Class 5 (0.5 ± 0.2 g). Final immersed weights were also significantly higher ($F_{(1,7)}$=26.04, p=0.001) in supplemented Class F6 than in Class 6. Although inital immersed weights were also higher in Class F6 (IW: 2.8 ± 0.8 g) than Class 6 (1.3 ± 0.2 g) ($F_{(1,8)}$=53.65, p=0.0001) initial contracted length, width and size index were not statistically different (p>0.050).

Prey-predator interactions

Juveniles

Juveniles of *S. endeca* were observed to prey on juveniles of *C. frondosa* under experimental conditions (Figure 2-11). Moreover, post-settled *C. frondosa*, never previously exposed to *S. endeca* expressed the typical escape behaviour of adults. In the experimental setup, two-thirds of newly introduced *S. endeca* (22 of the 32 individuals) moved directly towards the sea cucumbers and were able to position their bodies on top of their prey. Furthermore, 27 of the 32 tested *C. frondosa* displayed contraction and elongation whether or not they were touched by *S. endeca* during the exposure. Some individuals (n=7) even detached from the substrate and rolled away from the sea star in a successful escape. We were able to confirm that 13 of the 22 sea stars that moved toward the sea cucumbers succesfully captured them and partly evaginated their stomach before we ended the experiment. The other preying sea stars either lost the prey to a successful escape (n=5) or ceased their attack for unknown reasons (n=4).

Adults

Typical sea star behaviour during predation began with an approach where one to four of its arms nearest the sea cucumber were lifted upwards or backwards (Figure 2-12A, B). As the sea star came within reach of its prey, the arms would be brought downward in an effort to hold onto the sea cucumber as it positioned its mouth and body on top of it (Figure 2-12C, D). Also in 60 % of observations (n=41) *S. endeca* followed a circuitous route along the sides of the tank to "drop down" on sea cucumbers.

Cucumaria frondosa displayed escape responses when exposed to *S. endeca* independant of whether direct physical contact occurred. Introduction of the predatory sea star to the water prompted *C. frondosa* to increase their overall size and retract their tube feet and lay on the bottom of the tank (Figure 2-12E-H). As the sea star touched it with its arms, the unattached sea cucumber would be pushed away. Sea cucumbers also responded by undergoing a series of bloating and contracting motions when touched by a sea star making it difficult for *S. endeca* to enfold it (Figure 2-12E). If the sea star was able to place several arms or its body on top of the sea cucumber during a contraction phase, the sea star was pushed off as *C. frondosa* expanded, enabling it to escape. Failed attacks often left scrapes on the body wall of the sea cucumber. After cohabiting for a few days without successful predation, the sea cucumber resumed normal size, shape and feeding behaviour. However, additional physical contact from the sea star re-elicited an escape response.

Prey size selection and feeding cycle of adult *S. endeca*

A total of 16 small and eight large sea cucumbers were entirely or partially eaten over three months by six sea stars. Partial feedings by *S. endeca* (20 %) caused extensive damage to sea cucumbers, which ultimately died. In general, internal tissues were mainly consumed with remains left on the bottom of the tank. While medium-sized *S. endeca* (R: 11.5 ± 0.1 cm; WW: 382.9 ± 40.7 g) appeared to prey upon a higher number of small (CL: 6.4 ± 1.8 cm; IW: 0.6 ± 0.3 g) than large (CL: 14.2 ± 1.8 cm; IW: 5.8 ± 1.1 g) sea cucumbers, it was not statistically supported ($F_{(1,4)}$=0.471; p=0.530). No significant differences were observed between the sizes of sea cucumber eaten by large

(R: 14.5 ± 0.5 cm; WW: 621.5 ± 26.0 g) sea stars ($F_{(1,4)}$=1.00; p=0.374). Between sizes of sea stars, medium-sized *S. endeca* ate a higher number of small-sized sea cucumbers (4.0 ± 2.6) compared to large sea stars (1.0 ± 0.7) over the entire experiment; however this was not significantly different ($F_{(1,4)}$=0.125; p=0.742). The number of large sea cucumbers consumed by both sizes of *S. endeca* was low (medium sea star: 1.3 ± 0.6; large sea star: 1.0 ± 1.0) and the difference was not statistically different ($F_{(1,4)}$=0.000; p=1.000).

Feeding rates and fasting periods were dependant on the size of sea cucumber consumed (Figure 2-13). Medium sea stars partially consumed large-sized sea cucumbers and entirely consumed small-sized sea cucumbers over 1-4 days. Feeding upon a whole large-sized sea cucumber by a medium-sized *S. endeca* lasted 12 days and was followed by a fasting period of 12 days; however this was based upon one observation (Figure. 2-13). Fasting periods between feedings lasted 1-25 days, with an average of 9 ± 7 days for medium sea stars. Feeding bouts of large-sized *S. endeca* eating large *C. frondosa* lasted 9-13 days, whereas small-sized prey were eaten in a single day. Feeding ceased in large-sized sea stars for 1-14 days between bouts with an average fasting period of 7 ± 5 days. One large sea star only fed once during the entire experiment, and subsequently refrained from feeding for over 50 days (Fig. 2-12). Feeding also ceased in all large sea stars by mid-July as water temperatures reached ca. 10 °C. A similar cessation of feeding occurred later in medium sea stars as water temperatures peaked at 14 °C (Figure 2-13). All the sea stars, with the exception of a single mortality, had increased in size by 5-110 % by the end of the study. From June to July, medium *S. endeca* mainly consumed 2-4 small sea cucumbers per month and large individuals ate one large sea cucumber per month.

Feeding behaviour of *S. endeca* in relation to temperature

Feeding activity by the two sizes of *S. endeca* was confirmed experimentally to be affected by high temperature (Figure 2-14). A two-way ANOVA found a main effect of temperature ($F_{(1,45)}$=16.300; p=0.000) but not of size of *S. endeca* ($F_{(1,45)}$=1.167; p=0.286) indicating that sea stars were more likely to feed at lower temperatures. No significant interaction ($F_{(1,45)}$=0.340; p=0.568) was found between temperature and size of *S. endeca*. At low temperatures attacks on sea cucumbers occurred after an average of 1.3 ± 1.3 h in medium and 1.3 ± 1.6 h in large-sized *S. endeca* with 80-100 % of tested sea stars attacking sea cucumbers. At high temperature, the feeding response was 0.4 ± 0.1 h in medium and 1.7 ± 1.8 h in large-sized *S. endeca* where only 38-44 % of tested sea stars attacked sea cucumbers. There was no significant difference in feeding response time between temperature regimes ($F_{(1,29)}$=0.229; p=0.636) or sizes of sea star ($F_{(1,29)}$=1.221; p=0.278), nor any significant interaction between the two factors ($F_{(1,29)}$=1.467; p=0.236). All sea stars tested at night attacked sea cucumbers within 1.5 h at low temperature. Extreme low temperatures (-1 to 0 ^{o}C) did not prevent any size of *S. endeca* from feeding in the holding tanks (data not shown).

Reaction of *S. endeca* to damaged and intact prey

Of the 20 individuals of *S. endeca* (R: 13 ± 2 cm; WW: 608 ± 245 g) tested to determine their preference between an intact and damaged sea cucumber, 15 exhibited feeding behaviour (Figure 2-15). All 15 consumed the damaged individual first and within 24 hours. Moreover, by 48 hours, six sea stars secondarily attacked and/or ate an

intact individual (the second available prey in the tank). The feeding response time on damaged individuals was less than 3 h in 73 % of observed feedings, however the remaining sea stars had response times of over 20 h. Throughout the experiments, no escape response was ever observed in damaged sea cucumbers exposed to *S. endeca*. The experiment was repeated with *A. rubens,* but no attraction or feeding behaviour was observed (Fig. 2-14). The sea stars stayed in one area of the tank and showed no interest in feeding on either sea cucumber.

2.5 Discussion

Natural abundance

Contrary to predictions based on the distribution of the sea cucumber established by diving surveys (DFA 2002b; Hamel and Mercier 2008a), hard rocky substrates yielded the second lowest densities of all substrates sampled (rocky, gravelly, rocky-gravelly, and sandy substrates) by sea cucumber drags. However, the drags are towed along the sea floor and operate best on flat bottoms with few obstacles. Sampling in rocky environments fills the net with rocks and the drag may therefore miss sea cucumbers entirely due to the irregular nature of the bottom. The number of animals captured among tows was highly variable, either reflecting unequal efficacy of gear or the patchy distribution of *C. frondosa*. Areas of hard gravelly substrate yielded the highest densities of sea cucumbers in areas without its main predator, *S. endeca*.

Cucumaria frondosa densities were dependant on substrate type and region. Regionally, sea cucumber densities were higher offshore than inshore, regardless of

predator presence. Lower inshore densities may be resultant of more intense fishing as there are lower costs associated with fishing closer to land (Lew Barrett personal communication). Offshore areas may have more optimal environmental conditions for supporting higher densities of sea cucumbers. There was a significant interaction between region and presence of *S. endeca*. Offshore, there was no significant difference in sea cucumber density between areas with and without *S. endeca*. In the inshore region, presence of *S. endeca* was associated with higher densities of *C. frondosa* suggesting that sea stars are attracted to areas with high densities of sea cucumbers. Some areas showed occurrence of high numbers of each species, for instance over 630 sea cucumbers were caught in a single tow on a rocky-gravely substrate that yielded over 300 *S. endeca* as bycatch. Although chemodetection abilities vary between sea stars (Rochette et al. 1994) aggregations of *S. endeca* in areas with large numbers of sea cucumbers may indicate that they have the capacity to detect them. However, similarity in habitat preference may also account for this pattern and cannot be entirely ruled out.

Overall densities of sea cucumbers indicate a preference for hard substrates compared to soft sandy substrate. Similar distributions of *C. frondosa* have been observed in the Gulf of St. Lawrence (Canada) and off Maine (USA) with declining abundance from areas of hard bedrock and boulders to soft sand and mud (Himmelman and Dutil 1991; Kirshenbaum 2006). Higher densities may be attributed to larval settlement which selects for hard substrate that provides refuges for recruits (Hamel and Mercier 1996a, Medeiros Bergen and Miles 1997). Juveniles and adults also require hard surfaces for attachment which is necessary for feeding (Hamel and Mercier 1996a; Singh et al. 1998), presumably leading to better fitness and physical condition of the sea cucumber.

Effect of habitat on sea cucumber size

The morphometric analysis of sea cucumbers from various substrates revealed that individuals found on hard rocky substrate were larger than those found on either hard mixed or soft sandy substrate. One group of individuals from sandy substrate (sample S2) were the smallest of all sea cucumbers sampled. Increasing size with increasing hardness of substrate supports that rocky substrates are an optimal habitat for this species, as previously suggested (Hamel and Mercier 1996a). If sandy substrates do not provide adequate attachment for feeding, the presence of small sized individuals on soft substrate may be due to a resorption of tissues associated with low feeding rates, however feeding observations in the field and biochemical analyses are needed to confirm this. High stress due to rolling on the sandy bottom (CSAR 2006) may also contribute to reduced size. The higher gonadal index observed in some individuals from sand must be interpreted with caution as it may be attributed to a resorption process rather than higher reproductive potential. Since the gonad index is the ratio between gonad and body wall weight (including muscles), reduction of the latter would yield high values not indicative of gonad growth. *Cucumaria frondosa* is known to use energy from the body wall and associated muscles as an energy reserve to support gamete synthesis and maintain body functions during non-feeding periods (David and McDonald 2002). This would suggest that sea cucumbers on sand may be decreasing in size in response to low feeding and consumption of energetic reserves. The fact that sea cucumbers from sandy substrate generally had thicker body walls (excluding muscles) compared to those from mixed gravelly and rocky substrates may be the result of rolling along the sea floor, toughening

the wall, but only under the assumption that sea cucumbers spent enough time on this substrate. This highlights the inherent difficulties in comparing sea cucumbers subjected to different environmental conditions. Assuming that sea cucumbers are being displaced towards sandy substrates by currents or storms, there is no way of knowing how long they have been there prior to the collection. The lack of hard structures in sea cucumbers (Kirshenbaum et al. 2006) makes it impossible to separate age-related and environmentally-induced effects on reproductive potential. All these factors may contribute to the large variability noted in measures of sea cucumbers collected on sand. Long-term histological and field feeding studies are needed to support differences in body condition and its effect on reproductive potential of sea cucumbers from various substrates.

High selectivity for hard substrates is common in sea cucumbers (Woodby et al. 2000; Mendes et al. 2006). Sandy substrates do not provide firm attachment so that ocean currents would likely displace them with the sand to which they are attached (Woodby et al 2000; Džeroski and Drumm 2003). Fine particles may also be suspended with currents, inhibiting normal suspension feeding activities (Džeroski and Drumm 2003). Hard substrates on the other hand are rich in refuges and crevices, providing protection to small individuals from predators (Hamel and Mercier 1996a, Medeiros-Bergen and Miles 1997). They also provide firm attachment to adults for spawning. It is therefore legitimate to presume that individuals rolling on soft sand substrate will not contribute significantly to reproduction and recruitment.

Accuracy of measurements

The primary difficulty with sea cucumber growth studies has long been the reliability of measures used due to the plastic nature of the sea cucumber body. Although reliable measures such as dry weight have been developed (Rutherford 1973; Turner and Rutherford 1976; Hamel and Mercier 1996a; David and MacDonald 2002) they are obviously not suitable for growth experiments where individuals need to be kept alive and measured at regular intervals. Immersed weight is ideal for sea cucumber growth studies because it negates the effects of retained water within the organism, providing a measure of tissues as shown by the strong relationship with dry weight we obtained (r^2=0.97). Similar results have been obtained for *C. frondosa* and other echinoderms in previous studies (Grosjean 2001; Gudimova et al. 2004). Spirlet et al. (2000) developed a standardized immersed weight based on the salinity of the water in which the organisms were weighed. Since salinity changes off the coast of Newfoundland are minimal (ca. 32; Kendaris 1980), they were assumed to have negligible effect on the results of this study. While immersed weight is by far the most accurate measurement, it was also the most time consuming to use. Sea cucumbers needed to be completely covered by water and checked to ensure there was no contact with the sides of the seawater container which otherwise resulted in abnormally high values. It is doubtful that this measurement would be useful in the field as movements, such as the rocking of a boat, could also affect it. Wet weight was dependent on the amount of retained water within the animal, making it quite variable, yet it was still fairly well correlated with dry weight. As a sea cucumber's body size increases, the volume of water it is able to retain increases, accounting for the strong linear relationship. However, the drawback is that behavioural and physiological

processes unrelated to growth may also result in increased wet weight. Contracted length exhibited the poorest correlation with dry weight, however when combined with contracted width in the size index measure, the correlation was higher than for each measurement separately.

Growth of juveniles and adults

Spawning of *C. frondosa* took place in the laboratory from late March to early May in 2006 and 2007 after natural increases in phytoplankton levels. Hamel and Mercier (2008a) suggested that differences between regions in timing of phytoplankton blooms account for the wide variation of spawning times (Feburary to June) previously reported for *C. frondosa.* Similarly, the full size of mature oocytes in *C. frondosa* varies with geographical locations (Britt and Petersen 1982; Hamel and Mercier 1996a, 2008a), presumably as a result of differences in nutrient levels (Beddingfield and McClintock 1998) to which spawning adults are exposed. Larger oocytes have been observed in the St. Lawrence Estuary (0.7-1.1 mm; Hamel and Mercier 1996a) where high phytoplankton levels are present for 11 months of the year, compared to 3 months in the Newfoundland region (Afanasyev et al. 2001).

The smaller oocytes in our study metamorphosed into pentactula after ca. 15 days compared to ca. 17 days for the larger oocytes in a previous study (Hamel and Mercier 1996a). This trend is consistent with the observed correlation between egg size and development time in lecithotrophic larvae (Marshall and Bolton 2007). Small oocyte size indicates shorter dispersal distances with increased emphasis on local recruitment. Temperature variations during larval culture may also influence development time

(Hamel and Mercier 1996a; Hoegh-Guldberg and Pearse 1995) however temperature conditions were similar in both studies.

In the St. Lawrence Estuary, Hamel and Mercier (1996a) reported that settlement occurred after ca. 6 weeks, compared to ca. 5 weeks in our study. Faster settlement may enable new recruits to take advantage of the shorter productive season, however after two years of growth sea cucumbers reached a maximum of 6 mm in our study compared to over 30 mm in the St. Lawrence Estuary (Hamel and Mercier 1996a). The size of larvae at settlement was also smaller, a result that could be explained by the smaller oocyte size in Newfoundland populations (Marshall and Bolton 2007), and may contribute to the overall size differences after 24 months, although the coefficient of variation was higher for the final size of juveniles than it was for oocyte size.

It is unlikely that small larvae/juveniles were space-limited in our tanks as they occur in such high densities in the field (Hamel and Mercier 1996b), thus food level is the most likely cause of the lower growth rates recorded. Although sea cucumbers in our study were constantly supplemented with algae (3.8×10^5 cells ml^{-1}), the food availability was apparently not enough to enhance or even sustain growth rates. Furthermore, high variations in sizes were observed in juveniles of the same year class indicating differences in growth rate within a cohort reared in a common enclosure under identical environmental conditions. Larger individuals were generally found on the undersides of rocks possibly closer to detrital food sources, including accumulated organic material on the bottom of the tank. Sheltering under rocks also allows juveniles to feed uninterrupted for longer periods than in open areas where they are exposed to water currents and other disturbances. Similar observations of variable growth rate observed in the field were

attributed to differences in inter-seasonal growth rate, dependant on time of spawning (Medeiros-Bergen and Miles 1997). Intra-cohort size variations should definitely be given more consideration, especially since size is currently being used to estimate age and to establish cohorts (and sometimes infer spawning frequency) (Gage 1994), when in fact individuals of very different sizes could belong to the same cohort, and vice versa.

The growth patterns of *C. frondosa* in this study were consistent with seasonal feeding cycles previously detected in northwest Atlantic waters (Hamel and Mercier 1998; Singh et al. 1999). *Cucumaria frondosa* responds to high levels of phytoplankton in the water rather than to overall increases in organic material (Singh et al. 1999). Phytoplankton levels are generally low in the Newfoundland region with the exception of the spring phytoplankton bloom in April-May (Pomeroy et al. 1991; Afanasyev et al. 2001; Thompson et al. 2008; Wu et al. 2008). Sea cucumbers showed noticeably increased feeding during this time of year as shown by the full extension of their tentacles and their cyclic insertion into the mouth. In Passamaquoddy Bay, Singh et al. (1999) observed that *C. frondosa* feeding was minimal when phytoplankton levels were low. Similar growth trends were shown in most of the size classes tested here, with increases in immersed weight soon after spring blooms, owing to lag time in vertical flux of phytoplankton (Thompson et al. 2008) and then declines during the rest of the year. The seemingly rapid change in growth may also be attributed to the release of fatty oocytes from females during spawning, resulting in reduced buoyancy and increased immersed weight. Nevertheless, similar growth patterns were observed in other size measurements (data not shown). While overall size did not significantly change over the course of the study, Bonferroni adjusted comparisons are known to be conservative. In addition, the

similar size between replicates provides evidence that periodic sampling had minimal effects on growth. Minimal changes in size in the largest individuals was expected since they are close to the maximum size for the species in Newfoundland waters and are presumably undergoing slow or null growth. The seasonal decline in immersed weight in all size classes may be the result of resorption of reserves stored in the body wall (David and MacDonald 2002) due to starvation and low availability of food. In the field, annual increases in size likely occur with the spring phytoplankton bloom and declines in size may be lower. In general, it may be efficient for sea cucumbers to feed only during periods of high food levels, live on stored reserves during non-feeding periods and invest in reproduction. Singh et al. (1999) also proposed that short feeding periods could minimize tentacle cropping by fishes, a further argument against trying to feed when food is too low. Similar to what we report here, increases and decreases in size have been observed in other temperate and tropical holothurians to coincide with feeding and non-feeding periods (Rutherford 1973; Costelloe and Keegan 1984; Cameron and Fankboner 1989; Fu et al. 2006; Paltzat et al. 2008). Echinoderms can reabsorb tissues in response to low food levels, generally resulting in a reduction in size/weight (Ebert 1967), which adds to intrinsic difficulties in determining size-at-age. Size classes in the present study may thus have grouped individuals of different ages, and some of the variability in growth may be due to age-related metabolic differences (Guillou and Lumingas 1999). In turn, inter-individual variations in growth, as shown clearly in laboratory-reared juveniles, may mask individual growth patterns. Stocking density may also affect growth (Levitan 1988), however *C. frondosa* naturally occurs in high densities and 'shrinking' was observed even at very low densities in the laboratory. Uthicke et al. (2004) reported shrinking of

Holothuria nobilis in the field regardless of density and suggested that it was in response to unfavourable environmental conditions.

Sea cucumbers that were supplemented with algae during our study showed growth trends that were similar to those of individuals exposed to ambient food levels, although a significant decrease in the size of fed individuals was only observed in the smallest size classes. Seasonal size variations were not significant, suggesting that fed individuals were not as food limited as the others. Furthermore, fed individuals from one of the size classes were larger than the non-fed individuals at the end of the 17 months suggesting that they did not need to draw upon their energy reserves as much. The lack of significant growth in spite of the addition of algae, however, indicates that algal densities were insufficient to drive growth rates much higher. Alternatively, algae supplied may not have been suitable to meet the nutritional requirements of the sea cucumber; even though the algal species provided have been successfully used in previous laboratory feed studies with *C. frondosa* (Singh et al. 1998).

Overall growth was extremely limited during this 17-month study. An extrapolation of yearly growth rates from each size class of *C. frondosa* during peak phytoplankton levels suggests that growth to adult size (IW: ca. 6.5 g) would take a minimum of 25 years. This estimate is longer than previous estimate of ca. 10 years (minimum) from juvenile to market size (Hamel and Mercier 1996a, 2008; Gudimova et al. 2004). As already discussed, this marked difference most likely reflects the shorter growth season in northwest Atlantic waters, which is dependent on local phytoplankton levels (Afanasyev et al. 2001). The different methodologies used may also account for some of the variability. We used laboratory settings that mimicked field conditions

(running unfiltered seawater; naturally varying temperature and photoperiod) and chose immersed weight as a measure of sea cucumber size. In contrast, Hamel and Mercier (1996a) used *in situ* measures of length to establish growth rates of small *C. frondosa* in the field. Growth curves using contracted length in our study were similar to those constructed with immersed weight, although the changes in size were not as pronounced (data not shown). Gudimova et al. (2004) conducted a series of field and laboratory studies to elucidate growth of *C. frondosa*, using a less reliable measurement (total animal wet weight) to calculate parameters for their growth model. Our study showed no significant adverse effect of repeated measurements, except in size Class 3 where individuals sampled twice were double the immersed weight of individuals sampled nine times over 17 months. On the other hand, as mentioned previously, important size differences were also noted in laboratory-reared juveniles that were hardly ever disturbed, suggesting that genetic predisposition or physiological condition, rather than manipulation or stress, may affect growth rates. Growth rates in *C. frondosa* were also observed to be favoured by stable environmental conditions at certain depths (Hamel and Mercier 1996a), further indicating that any measure of growth must be interpreted cautiously.

Growth to maximum size in echinoderms is often quite slow (ca. 40 years to adult size in *Sterechinus neumayeri*; Brey et al. 1995). Because animals are rarely monitored for more than a few years (Rutherford 1973; Herrero-Perézrul et al. 1999; Uthicke et al. 2004, Paltzat et al. 2008), growth models are typically used to extrapolate growth to maximum size (Herrero-Perézrul et al. 1999; Grosjean 2001). Assuming that measurements accurately reflect size, the lack of size-at-age data for older individuals can

lead to biases in growth estimation. Furthermore, growth models presume that size and age are directly related, which is not the case for many echinoderms (Ebert 1967). The cyclic growth and shrinking of sea cucumbers, combined with intra-cohort size variations, can further confound the establishment of proper size classes to estimate growth. On the other hand, tagging individuals for long-term studies is difficult (Kirshenbaum et al 2006; Shiell 2006) and holding them in groups is crucial for reproductive fitness due to inter-individual gametogenic cues (Hamel and Mercier 1996c; 1999a). Thus, in spite of its possible shortcomings, our growth study used one of the most accurate measures of sea cucumber size and was effective in showing the seasonal food-related nature of growth of *C. frondosa*. Ideally, studies should be conducted in the field to determine natural growth and shrinking rates of individuals using DNA fingerprinting (Uthicke et al. 2004) to further refine our estimates.

Predation by sea stars

This study marks the first observation of predation on juveniles of *C. frondosa* by juveniles of *S. endeca*. One year old juveniles of *C. frondosa* reared in the laboratory exhibited the adult escape behaviour first described by Legault and Himmelman (1993), suggesting that the species is genetically predisposed to avoid its sea star predator, and that the latter develops predatory abilities early in its life. The innate ability of captively-reared juveniles for co-detection is evidence that these two species have likely co-evolved. Similar anti-predatory responses have been observed in juvenile *Parastichopus californicus* in the presence of adult *Pycnopodia helianthoides* (Cameron and Fankboner 1989). Accounts of predation on invertebrate juveniles have generally

been limited to incidental observations, however our study shows that juvenile predation could add considerably to the already high mortality rates of larval and small-sized sea cucumbers (Hamel and Mercier 1996a). Although encounter rates between juveniles of *S. endeca* and *C. frondosa* are potentially high as the sympatric species share similar spawning periods and larval ecology (Gemmill 1912; Britt and Petersen 1982; Medeiros-Bergen et al. 1995) differences in observed distribution may reduce predation impacts.

Well developed prey-predator interactions were observed between adults of *S. endeca* and *C. frondosa,* the former employing direct and indirect attacks where it would either move directly towards its prey, or take a circuitous route. Direct attacks were only observed if *S. endeca* was in close proximity to its prey. Indirect attacks occurred more often with the sea star positioned on the side of the tank, above its prey. Attacking from the side of the tank may allow sea stars to remove themselves from direct water flow, letting them approach from "down wind" without being sensed by the sea cucumber, a tactic similar to that of the sea star *Leptasterias polaris* (Rochette et al. 1994). It may also be a strategy to use their entire body weight to help capture the sea cucumber. Furthermore, predation experiments in a tank confined the sea cucumbers preventing them from escaping as successfully as they might in nature. Thus, due to the repeated attacks on large sea cucumbers, effective predation rates observed on large sea cucumbers in the laboratory may be higher than in the field.

In the confines of the tank, sea cucumbers exposed to non-feeding *S. endeca* for several days became habituated to their presence, returning to their normal size and eventually feeding. Anti-predatory responses were re-elicited if the sea star touched the sea cucumber. A similar behaviour was observed in *P. californicus* where active sea stars

prompted anti-predatory responses, however inactive sea stars, though in close proximity to the prey, did not (Cameron and Fankboner 1989). Quick contraction and elongation behaviours are presumably energetically expensive for *C. frondosa*. Since they live sympatrically with *S. endeca*, there must be a mechanism to recognize whether there is a threat or not, possibly through the chemical signature of "hunting/hungry" predators, as described by Cameron and Fankboner (1989).

The feeding cycle of adult *S. endeca* on *C. frondosa* revealed a preference for prey smaller than themselves and predation rates that varied with temperature. There seemed to be a preference for small sea cucumbers by medium-sized sea stars, although it was not statistically supported. No preference was observed in large-sized seas stars, however lower feeding rates may be due to their larger energy reserves and ability to consume prey with higher energy content. One sea star ceased feeding for 50 days with no noticeable negative effects except for minimal growth. Asteroids in general display a capacity to fast for long periods without any detriment, as shown by experiments with *L. polaris* starved for two months (Rochette et al. 1994). Feeding bouts were not dependant on size of the predatory sea star but on the size of prey item. Small-sized sea cucumbers were consumed much more quickly than were larger ones. Overall, the length of time spent eating was high, lasting up to 13 days, similar to observations in the field (Himmelman et al. 2005). Himmelman et al. (2005) attributed the long consumption times of the sea cucumber *Psolus fabricii* by *S. endeca* to the digestion of the tough body wall containing hard calcareous plates. *Cucumaria frondosa* are generally larger than *P. fabricii* and also have a tough body wall (as emphasized by the remains of partially eaten sea cucumbers consisting mainly of this tissue), leading to similarly long feeding bouts.

The escape behaviour of *C. frondosa* can be quite effective in foiling sea stars, especially against smaller predators. Although large sea cucumbers used in this study represent a larger quantity of food, they would be much harder to capture. Sea cucumbers smaller than the sea stars would be easier to capture, but higher quantities would need to be consumed to match the nutritional benefits of feeding upon a larger specimen. The feeding behaviour of *S. endeca* is similar to that of other sea stars which choose a prey size that increases their net caloric intake (Feder and Christensen 1966; Menge 1972; Paine 1976; McClintock and Lawrence 1985; Beddingfield and McClintock 1993) with respect to energy expended capturing prey and energy gained from it.

All *S. endeca* ceased feeding in late July 2007, presumably because temperature in the tanks reached ca. 14 °C, the upper limit of their tolerance level as estimated by Ursin (1960). Large *S. endeca* stopped feeding earlier, when temperatures reached ca. 10 °C, and fasted for about two months. Metabolic costs may be higher for large versus medium-sized sea stars at higher temperatures, inducing them to cease feeding at lower temperatures. Medium-sized sea stars could also have lower energy reserves than larger sea stars and thus a lower fasting capacity, increasing their need to feed at intermediate temperatures (10-13°C). Temperature-related experiments of feeding behaviour in *S. endeca* confirmed a marked preference for attacking sea cucumbers at lower temperatures. Contrary to feeding cycle observations, there were no significant size-related differences in feeding activity in relation to temperature detected in other sea stars (Feder and Christensen 1966). Temperature extremes have been known to minimize predation rates in *Pisaster ochraceus* (Sanford 2002a) and our study confirms that temperature is an important factor controlling sea star distribution and predator-prey

dynamics as previously suggested (Franz et al. 1981; Sanford 2002b). The mortality of one medium *S. endeca* near the end of the study is further evidence that 14 °C is close to thermal limit for this species.

Bottom trawls used for harvesting sea cucumbers, sea urchin and scallops are known to have devastating effects on benthic invertebrates and habitats (Hamel and Mercier 1999b; Veale et al. 2000a; Jennings et al. 2001; Robinson et al. 2001). Soft-bodied invertebrates like sea cucumbers may be crushed when rocks are flipped over or damaged when the sled-like rails on the bottom trawl run over them. The pair-wise prey preference experiment revealed an inclination of *S. endeca* towards damaged sea cucumbers over intact ones. Damage to the body wall of the sea cucumber, exposing internal organs, prevents it from effectively employing anti-predator behaviours. Secondary intentions were observed in ca. 40 % of the cases (6 of 15) where sea stars feeding on damaged sea cucumbers subsequently attacked and ate intact individuals. Five out of the six observations of secondary attacks were carried out by large *S. endeca*, confirming the need for increased food intake. Previous studies on scallops *Chlamys islandica* have shown that sea stars are attracted to recently fished areas due to the increase in damaged scallops and thus an increase in availability of food (Veale et al. 2000b). A similar attraction of *S. endeca* is likely present in areas where sea cucumbers are fished due to the abilities of sea stars to detect *C. frondosa* and their capacity to travel distances >5 m in 12 h (Himmelman and Dutil 1991). Although the sea star *Asterias rubens* is an opportunistic scavenger and has been reported to consume *C. frondosa* in the laboratory (Coady 1973) no feeding response the was observed in our study, although

other invertebrate scavengers have been observed to feed upon *C. frondosa* damaged by trawls (Robinson et al. 2001).

The population structure of *S. endeca* on the St. Pierre Bank is comparable to that measured in other areas of its distribution range (Zolotarev 2002). Medium-sized sea stars (ca. R: 7-11 cm) account for up to 57 % of the population, suggesting that small individuals of *C. frondosa* (IW: <2.0 g) would predominantly be targeted by predation. Larger, deep-water sea cucumbers are more likely to escape predation, however if they are damaged by bottom trawls, as observed in other fisheries (Robinson et al. 2001), their ability to escape may be drastically reduced. An extrapolation of overall predation rates based on the average size of *S. endeca* and assuming cessation of feeding during peak temperatures from August to October indicates that 2 % (0.012 ind m^{-2}) of sea cucumbers could be removed annually. This rate assumes monospecific predation on *C. frondosa*. It does not take into account predation on other echinoderms which can be commonly found in coastal waters (Gosner 1978; Himmelman 2006). For instance, Himmelman (1991, 2006) observed *S. endeca* generally feeding on *P. fabricii* and to a lesser degree on *P. phantapus* and *Chiridota laevis* in the St. Lawrence Estuary. On the other hand, *C. frondosa* is known to have other predators besides *S. endeca*, including fishes (Templeman 1982; Francour 1997; Hamel and Mercier 2008a). Furthermore, temperature on the St. Pierre Bank at depths where sea cucumbers are fished never increase above 6 °C (Drinkwater et al. 1999), suggesting that *S. endeca* can feed continuously.

Implications for the fishery

This study highlights aspects of the biology of *C. frondosa* that have major implications for a commercial fishery in northwest Atlantic waters. *Cucumaria frondosa* occurs in relatively high abundance but exhibits a patchy distribution in some areas that may translate into the possibility of increasing the fishing effort. Stock assessments of *C. frondosa* on the St. Pierre Bank are conducted using a sea cucumber drag prior to the start of the fishery to monitor eventual impacts on fished populations (L. Barrett personal communication). Surveyors weigh the total number of sea cucumbers caught and count and weigh any bycatch. Current methods of sampling may lead to biases in determining the biomass of *C. frondosa*, as the drag does not work optimally on rocky irregular substrates, potentially under-estimating sea cucumber densities. Addition of camera tows or divers to conduct transects may be used to supplement current stock estimates to improve accuracy.

Distribution and morphometric evidence also suggest that sea cucumbers are easier to trawl on sand, but they may yield a lower price as they are generally smaller in size. However, the increased thickness of the body wall may be beneficial for making beche-de-mer (Hamel and Mercier 2008). Because they appear to contribute less to recruitment in general, preferentially harvesting *C. frondosa* on sandy bottoms would presumably reduce impacts on reproductively important populations and the resource in general.

Growth rates of *C. frondosa* around Newfoundland are slower than previously reported for the species in estuarine habitats (Hamel and Mercier 1996a) and much slower than growth rates reported for tropical species of sea cucumbers (Herrero-Pérezrul et al.

1999). Even with good reproductive success (Hamel and Mercier 1996d) and large virgin biomasses, adults removed by the fishery will likely take many decades to replace, especially if predation pressure is mainly targeted at juvenile and small-sized sea cucumbers. There is a definite concern that predation impacts will also be exacerbated by fishing pressure whether it is sea cucumber-related or not. Harvesting pressure upon adults and predation pressure upon juveniles make sea cucumber populations extremely susceptible to Allee effects, reducing their ability to recover from overexploitation (Uthicke 2004). Deep-water refuges for sea cucumbers (>50 m) that are less accessible to harvesters have been cited as a potential recruitment source for fished coastal populations (Hamel and Mercier 1999b, 2008a). However, removal of coastal populations can reduce reproductive potential, as deep-water populations living beyond the photic zone may rely on cues from shallower populations to synchronize gametogenesis (Hamel and Mercier 1996c). Thus, removal of sexually mature coastal sea cucumbers potentially has impacts on a larger proportion of the reproductive population. Using a trawl to fish sea cucumbers may unintentionally help preserve reproductive adults and sources of recruitment as they are ineffective in irregular rocky environments, the preferred habitat of *C. frondosa* (Hamel and Mercier 1996a, 2008a).

Another factor that may help protect the sea cucumber populations from overfishing is the relatively low market value of *C. frondosa* (Therkildsen and Petersen 2006; Hamel and Mercier 2008a). In Indo-Pacific regions, populations of prime commercial species were overfished due to their high value and low harvesting costs (Uthicke and Conand 2005). In the northwest Atlantic, harvesters will likely shift to other fisheries should the fishery for *C. frondosa* become economically unsustainable.

Unfortunately, the sea cucumber population may be beyond the point of recovery should the fishery ever reach this stage. Already, populations of *C. frondosa* in Maine (USA), subject to the oldest fishery for this species, are showing signs of decline (Gudimova et al. 2004; Bruckner 2005; Hamel and Mercier 2008a).

Biological and ecological data suggest that *C. frondosa* is susceptible to overexploitation and Allee effects in a manner similar to other commercial sea cucumbers, regardless of the large initial biomass. A sustainable fishery can only be developed in Atlantic Canada if managers take a precautionary approach, harvesting a small percentage of the biomass and continuing to monitor and study *C. frondosa* populations in Atlantic waters. It is imperative to heed the lessons learned from overexploited sea cucumber fisheries elsewhere, and continue to have the support and cooperation of harvesters, processors and research scientists.

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2.8 Tables

Table 2-1: Mean measurements of whole specimens of *Cucumaria frondosa* from various substrates on the St. Pierre Bank. The number of specimens in each set is indicated (n) and standard deviation is noted in brackets. See text for description of measures and substrate.

Substrate	Group	n	Length (cm)	Width (cm)	Wet weight (g)	Size index	Split weight (g)
Hard (rocky)	R1	17	14.3	8.4	370	12.1	241
			(2.7)	(1.3)	(138)	(3.9)	(50)
Hard mixed (rocky and gravelly)	G1	21	14.2	6.2	212	8.9	116
			(2.0)	(1.0)	(68)	(2.4)	(21)
	G2	15	14.9	6.7	242	10.1	119
			(1.3)	(0.8)	(48)	(1.7)	(23)
Soft (sandy)	S1	23	15.0	5.8	198	8.8	122
			(1.3)	(1.0)	(64)	(1.8)	(21)
	S2	28	11.9	5.3	142	6.4	99
			(1.6)	(0.9)	(48)	(1.6)	(25)

Table 2-2: Description of main substrate types on the St. Pierre Bank, Newfoundland and Labrador.

Substrate hardness	Substrate type	Bottom	Composition
Hard	Rocky	Irregular	Bedrock bottoms with outcrops and boulders
	Gravelly	Flat	Small rocks (2-5 cm in diameter), shells, and tests of other invertebrates
Hard mixed	Mixed	Irregular	Combination of rocky and gravelly substrate
Soft	Sandy	Flat	Sandy bottom with scattered rocks (10-15 cm in diameter)

Table 2-3: Initial size classes used for the growth study of *Cucumaria frondosa* in the laboratory. Food supplemented classes are noted with an "F". IW represents immersed weight; WW represents wet weight; CL represents contracted length and CW represents contracted width.

Class	Replicates	n	IW (g)	WW (g)	CL (cm)	CW (cm)
1	1		-	-	0.075[a]	-
2	2	10	0.017 (0.018)	0.4 (0.2)	1.0 (0.4)	0.6 (0.1)
3	2	39	0.226 (0.079)	10.8 (4.1)	3.3 (0.8)	2.4 (0.5)
4	4	43	0.390 (0.162)	26.0 (10.3)	4.8 (0.6)	3.1 (0.5)
F4	2	10	0.349 (0.165)	28 (12)	4.7 (0.7)	3.3 (0.6)
5	4	20	1.28 (0.35)	121 (26)	7.6 (0.7)	5.5 (0.5)
F5	2	10	1.09 (0.17)	92 (29)	7.2 (0.6)	4.7 (0.5)
6	4	20	2.43 (0.79)	152 (40)	10.3 (1.0)	5.4 (0.7)
F6	2	10	4.04 (0.83)	264 (146)	11.8 (9.2)	6.2 (0.9)
7	4	20	5.34 (1.12)	265 (41)	13.3 (0.7)	6.3 (0.5)
8	4	20	8.20 (1.39)	584 (124)	16.8 (1.5)	8.5 (1.0)

[a] Size Class 1 was obtained from spontaneous spawning in the laboratory; initial size therefore corresponds to the mean size of fertilized oocytes.

Table 2-4: Development of *Cucumaria frondosa* in the laboratory. A new stage was established when ca. 50 % of embryos reached it. Larvae were reared under ambient photoperiod and temperature (-1 to 0 °C).

Stage	Size (mm)	Time
Just spawned oocyte	0.7 ± 0.1	0
Fertilized oocyte (elevated fertilization membrane)	0.73 ± 0.16 (excluding space between egg and membrane)	6 ± 2 min
2-cell	0.8 ± 0.1	5.1 ± 0.9 h
4-cell	0.8 ± 0.1	7.3 ± 1.1 h
Morula	0.8 ± 0.1	22 ± 2 h
Blastula (fully formed)	0.9 ± 0.1	41 ± 4.2 h
Early gastrula	0.8 ± 0.1	65 ± 6 h
Elongated gastrula	1.5 ± 0.2	4 ± 1 d
Vitellaria	1.5 ± 0.3	7 ± 1 d
Early pentactula (5 tentacles)	1.2 ± 0.2	10 ± 2 d
Late pentactula (5 tentacles and 2 podia)	1.3 ± 0.2	15 ± 1.5 d
Settlement (negatively buoyant, no more cilia)	1.3 ± 0.2	ca. 4.9 weeks

Table 2-5: One way ANOVA of immersed weight between replicates of *Cucumaria frondosa* in size Classes 4-8. See Table 2-2 for size class details.

Class	Source of variation	DF	SS	MS	F	P
3	Between groups	1	0.026	0.026	18.42	0.002
	Residual	10	0.014	0.001		
	Total	11	0.040			
4	Between groups	3	0.034	0.011	2.38	0.085
	Residual	36	0.17	0.0048		
	Total	39	0.21			
5	Between groups	3	0.20	0.067	1.69	0.208
	Residual	16	0.63	0.039		
	Total	19	0.83			
6	Between groups	2	0.081	0.041	0.45	0.647
	Residual	11	0.99	0.090		
	Total	13	1.07			
7	Between groups	3	2.55	0.85	0.68	0.580
	Residual	14	17.53	1.25		
	Total	17	20.08			
8	Between groups	3	1.33	0.44	0.65	0.597
	Residual	14	9.53	0.68		
	Total	17	10.86			

2.9 Figures

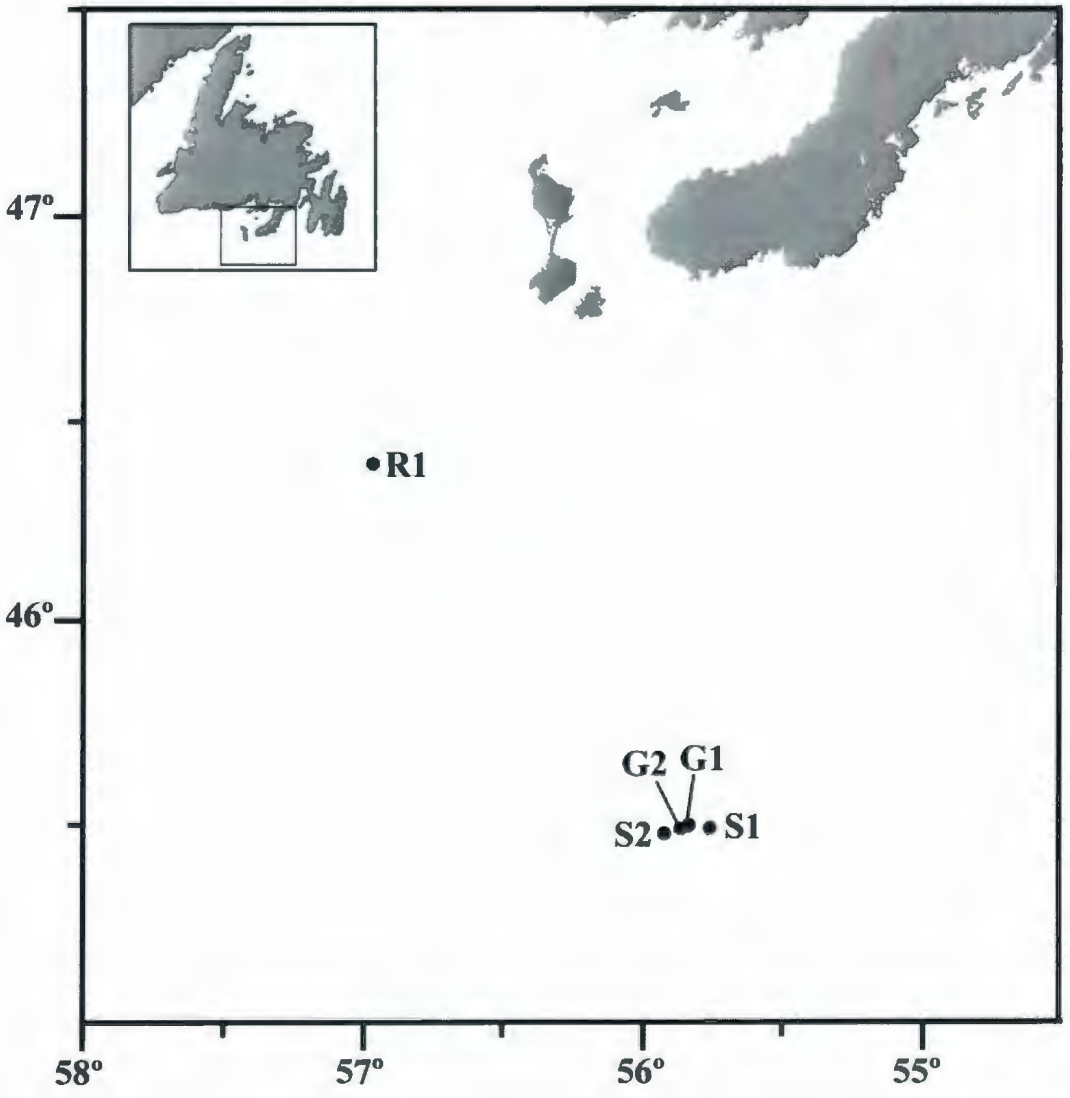

Figure 2-1: Sample sites for determining substrate related condition in *Cucumaria frondosa* on the St. Pierre Bank. Samples were collected from rocky (R1), gravelly (G1 and G2) and sandy (S1 and S2) substrates. See text for descriptions of substrates.

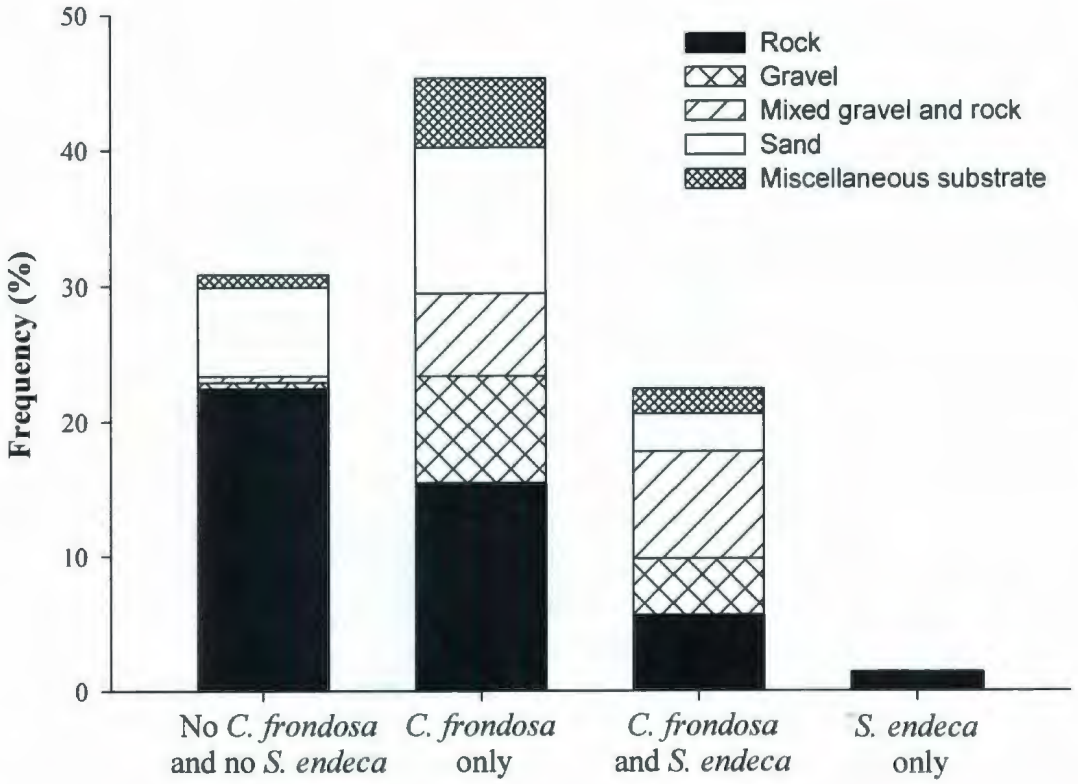

Figure 2-2: Frequency of substrate type relative to the presence or absence of *Cucumaria frondosa* and *Solaster endeca*. Miscellaneous substrate is comprised of a mixture of other substrates including rock, gravel, sand and shell.

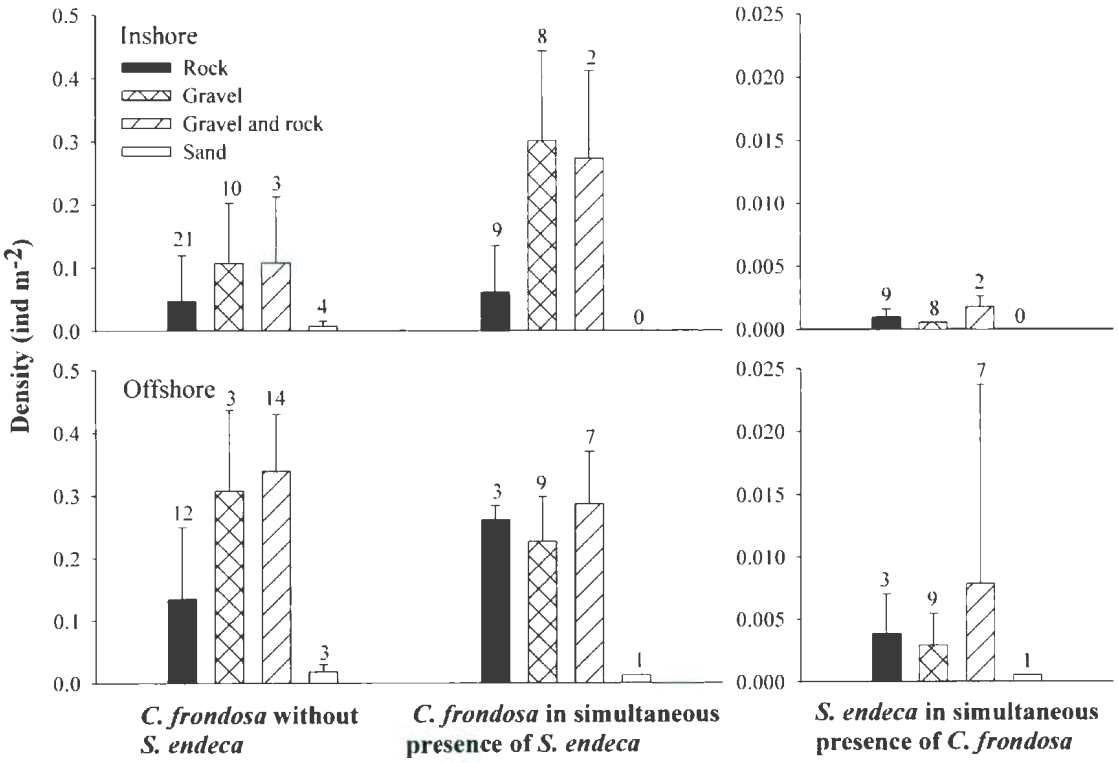

Figure 2-3: Densities of *Cucumaria frondosa* in areas where the predator *Solaster endeca* is present or absent on the St. Pierre Bank, Newfoundland and Labrador (Canada) determined by survey trawls in 2007 and densities of *S. endeca* in the presence of *C. frondosa*. There was insufficient data to determine densities of *S. endeca* in areas devoid of *C. frondosa*. Error bars indicate standard deviation and number of tows is indicated above the bars.

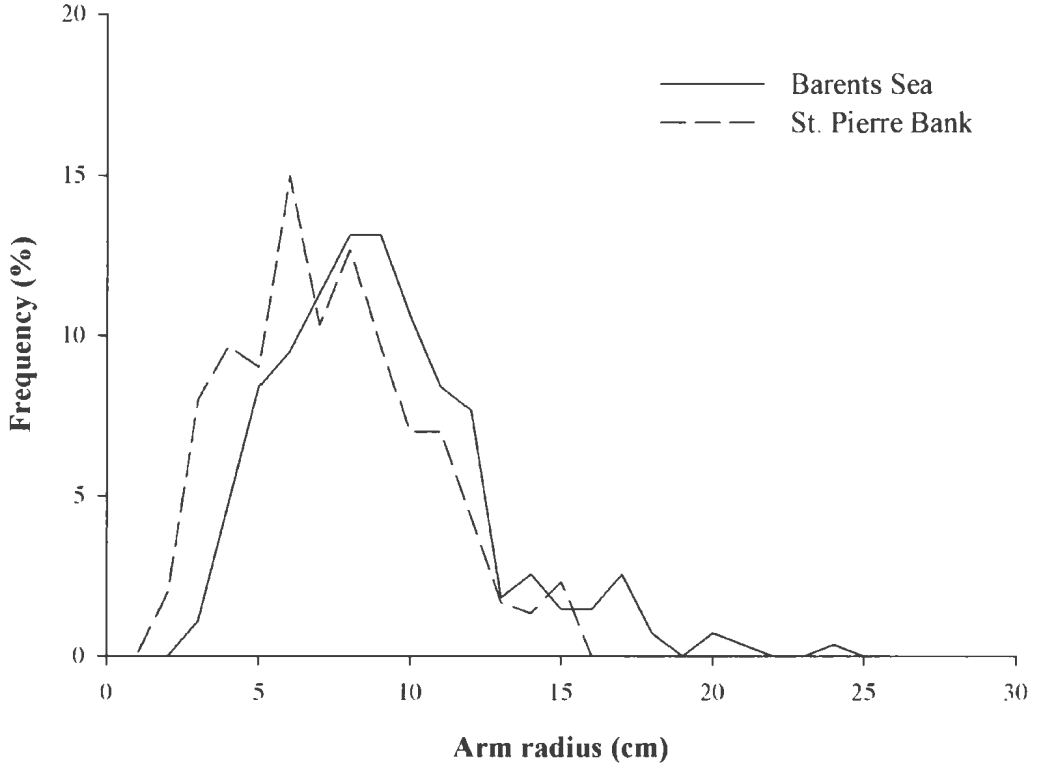

Figure 2-4: Average population size structure of *Solaster endeca* from the Barents Sea (1997-1999; 68°36'N, 40°00'E; data from Zolotarev 2002) and from the St. Pierre Bank (2006-2007; 45°46'N, 56°05'W; data from present study).

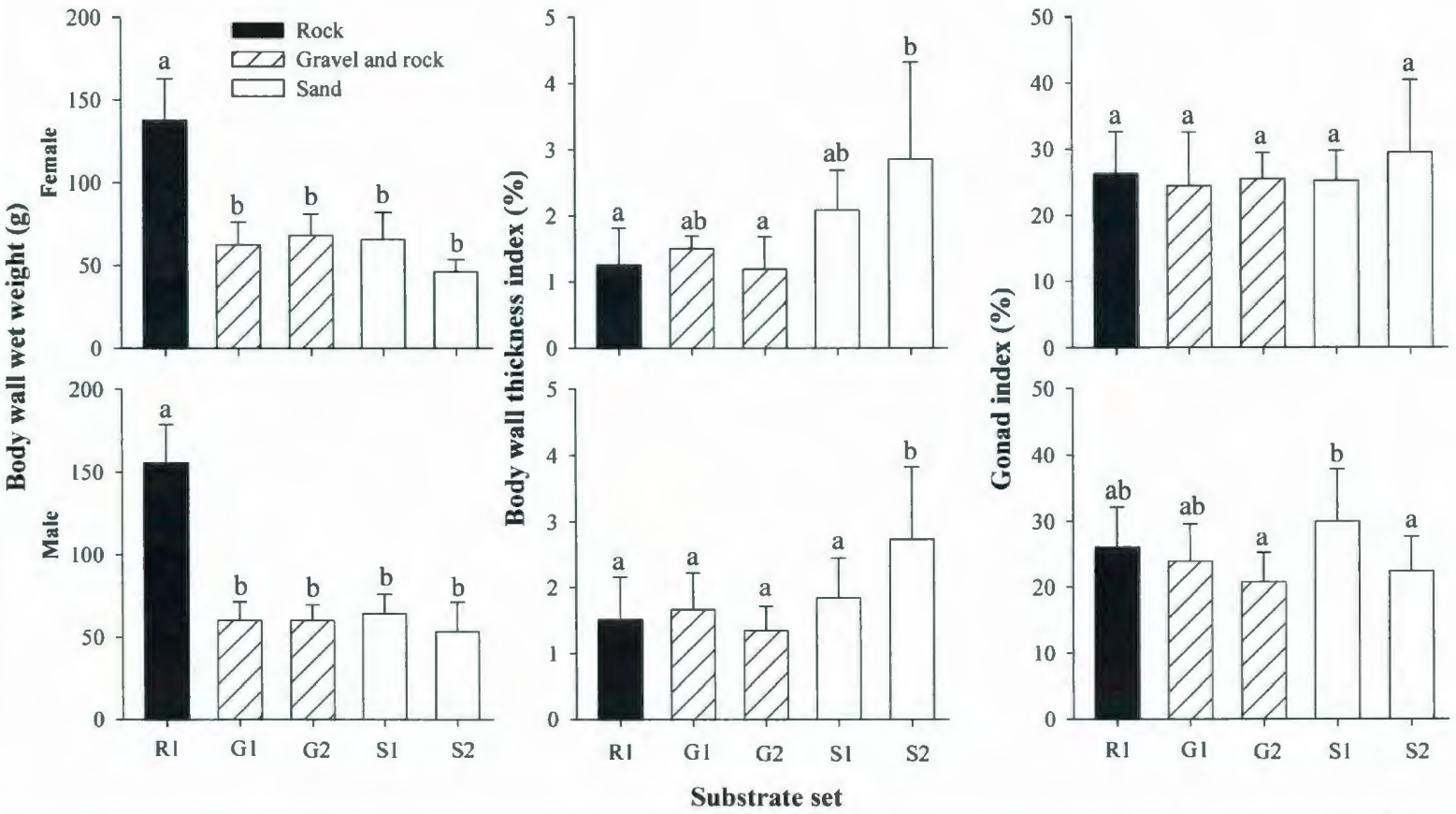

Figure 2-5: Average measurements of *Cucumaria frondosa* from rock (R1), mixed gravel and rock (G1 and G2) and sand (S1 and S2) substrate from the St. Pierre Bank. Error bars indicate standard deviation. Means with the same letter within the same graph are not significantly different (Bonferroni, α=0.05). See text for substrate descriptions and Figure 2-1 for location.

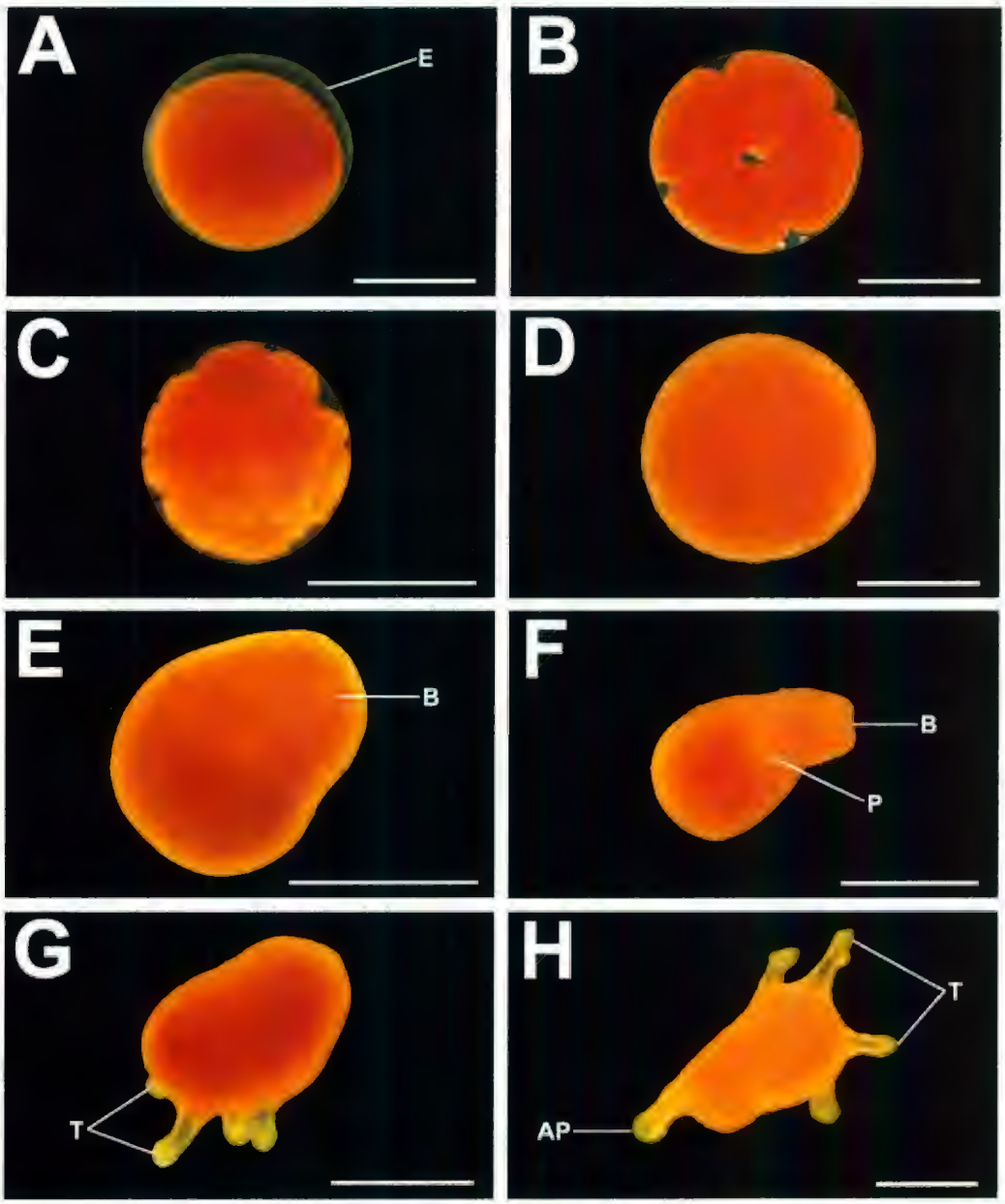

Figure 2-6: Principal stages of development in *Cucumaria frondosa* in the laboratory. A) Fertilized oocyte showing elevated fertilization envelope. B) 4-cell stage, C) 32-cell stage, D) blastula, E) young gastrula, F) vitellaria showing podial pit, G) early pentactula showing five tentacles, H) late pentactula showing a pair of ambulacral podia. E: elevated fertilization envelope; B: blastopore; P: podial pit; T: tentacles; AP: ambulacral podia. Scale bars represent 0.5 mm.

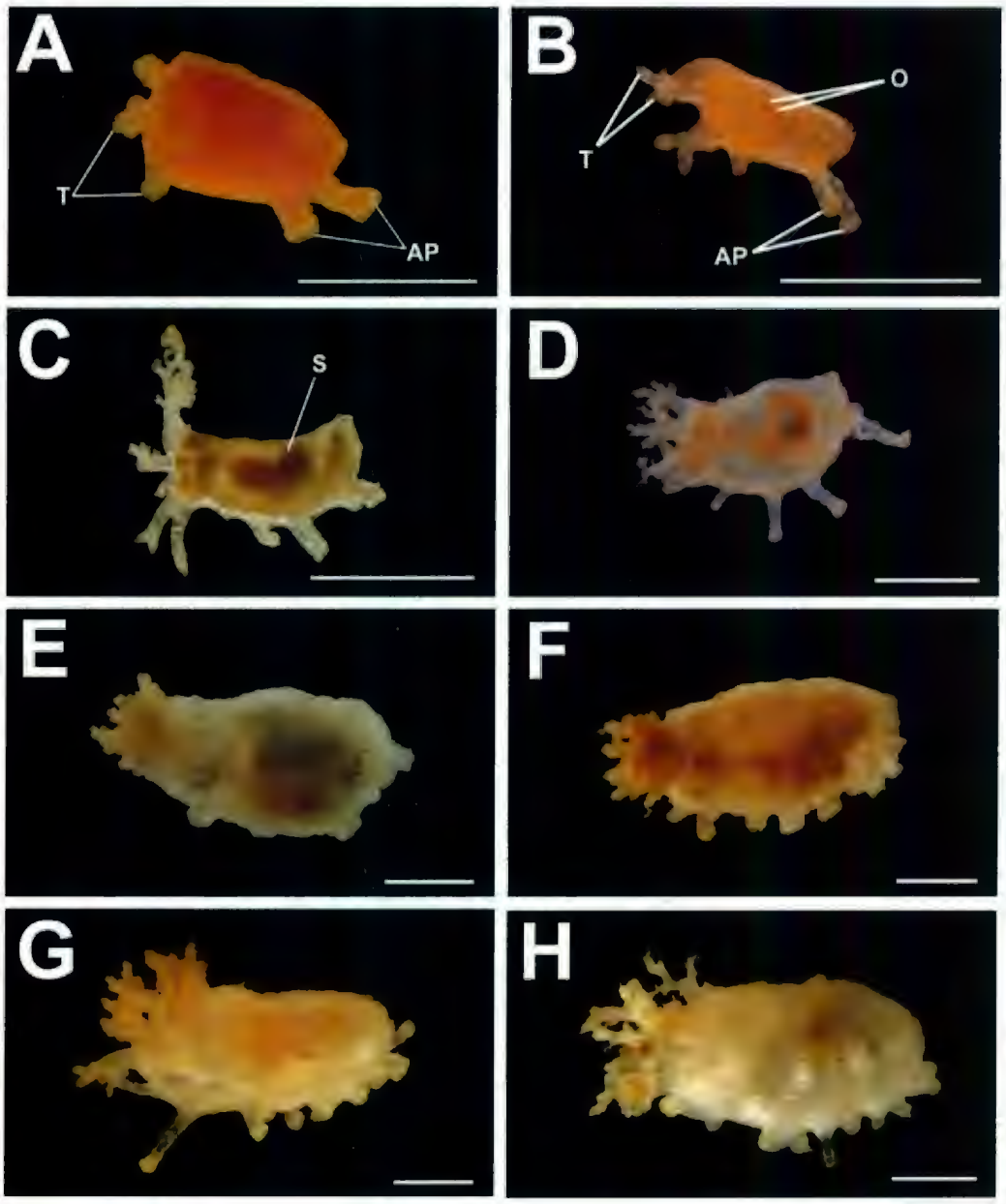

Figure 2-7: Development of juveniles of *Cucumaria frondosa*. A) two month old juvenile with first pair of podia, B) three month old juvenile showing ossicles and two pairs of podia, C) four month old juvenile showing stomach, anus and aquapharyngeal bulbs, three pairs of podia and the tentacles becoming ramified, D) six month old juvenile with four pairs of podia, E) 12 month old juvenile with five pairs of podia, F) 19 month old juvenile with eight pairs of podia, G) 22 month old juvenile with nine pairs of podia, H) 25 month old juvenile with ten pairs of podia. T: tentacles; AP: ambulacral podia; O: ossicles. Scale bar represents 1 mm.

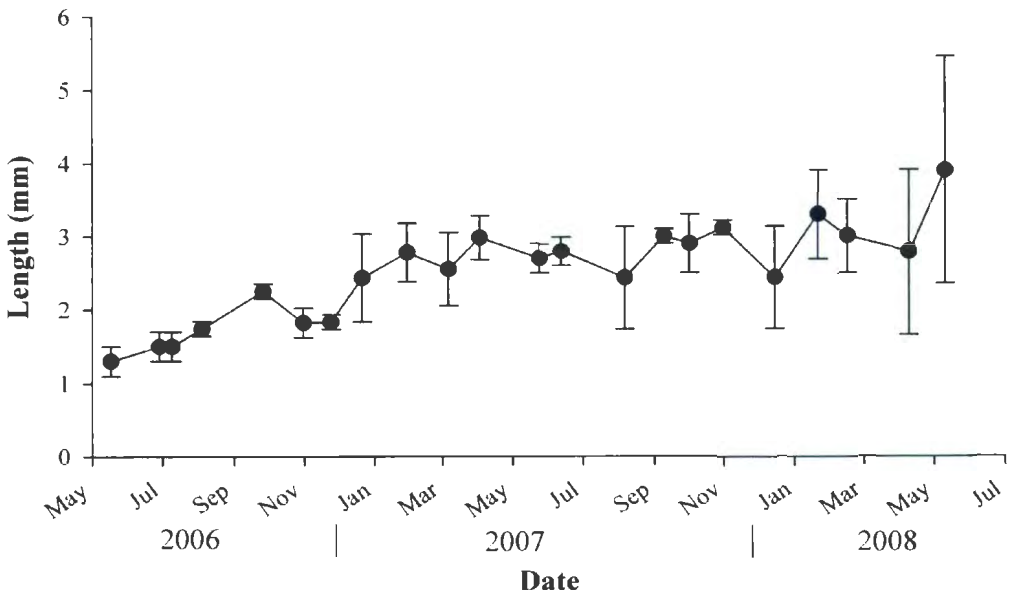

Figure 2-8: Monthly average length of size Class 1 juveniles of *Cucumaria frondosa* obtained from spontaneous spawning and reared in the laboratory from May 2006 to May 2008. Error bars indicate standard deviation.

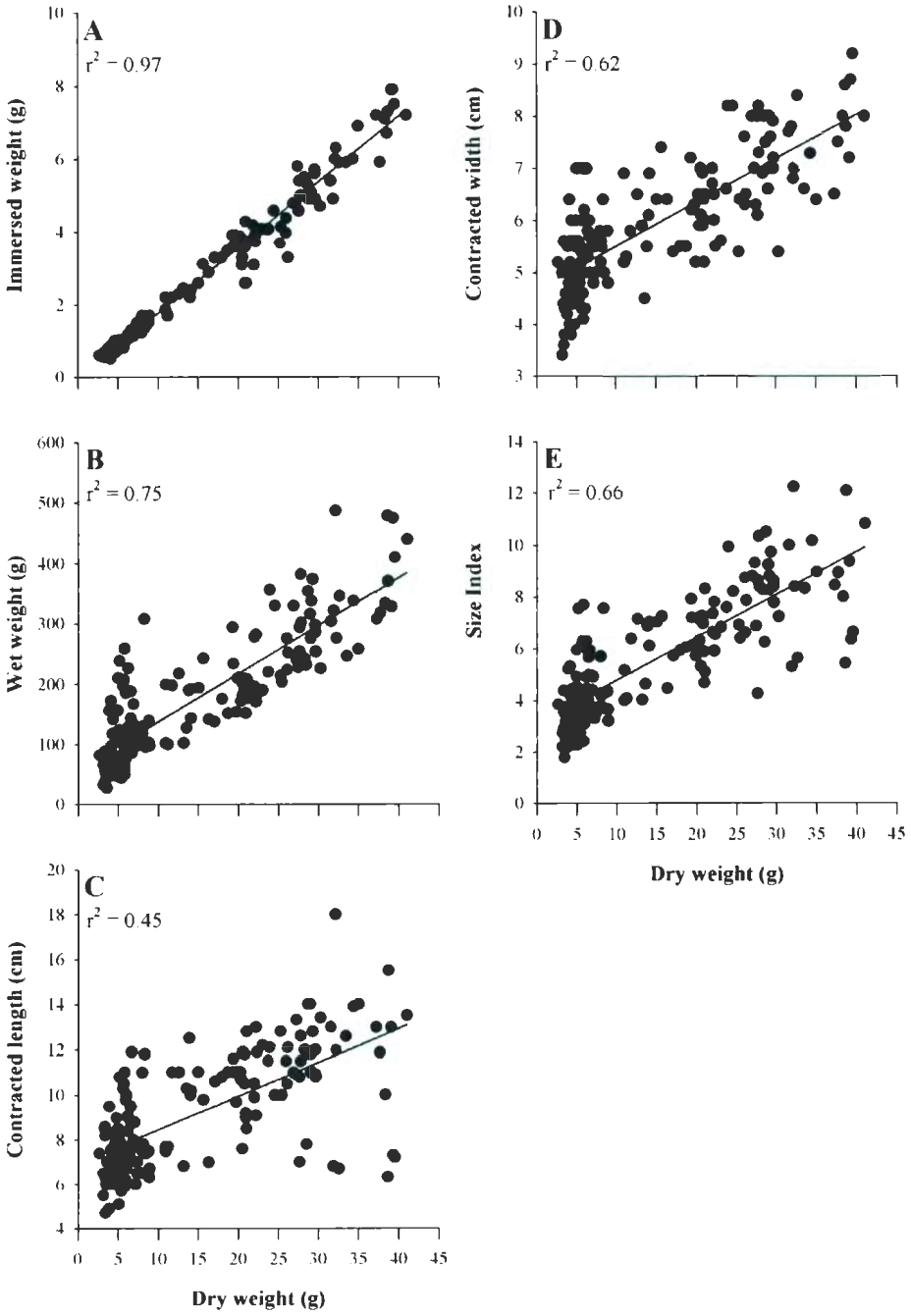

Figure 2-9: Morphometric measurements of *Cucumaria frondosa*. Relationship between dry weight and A) immersed weight, B) wet weight, C) contracted length, D) contracted width and E) size index. Fitted regression lines ($p<0.0001$) and coefficients of determination (r^2) are shown.

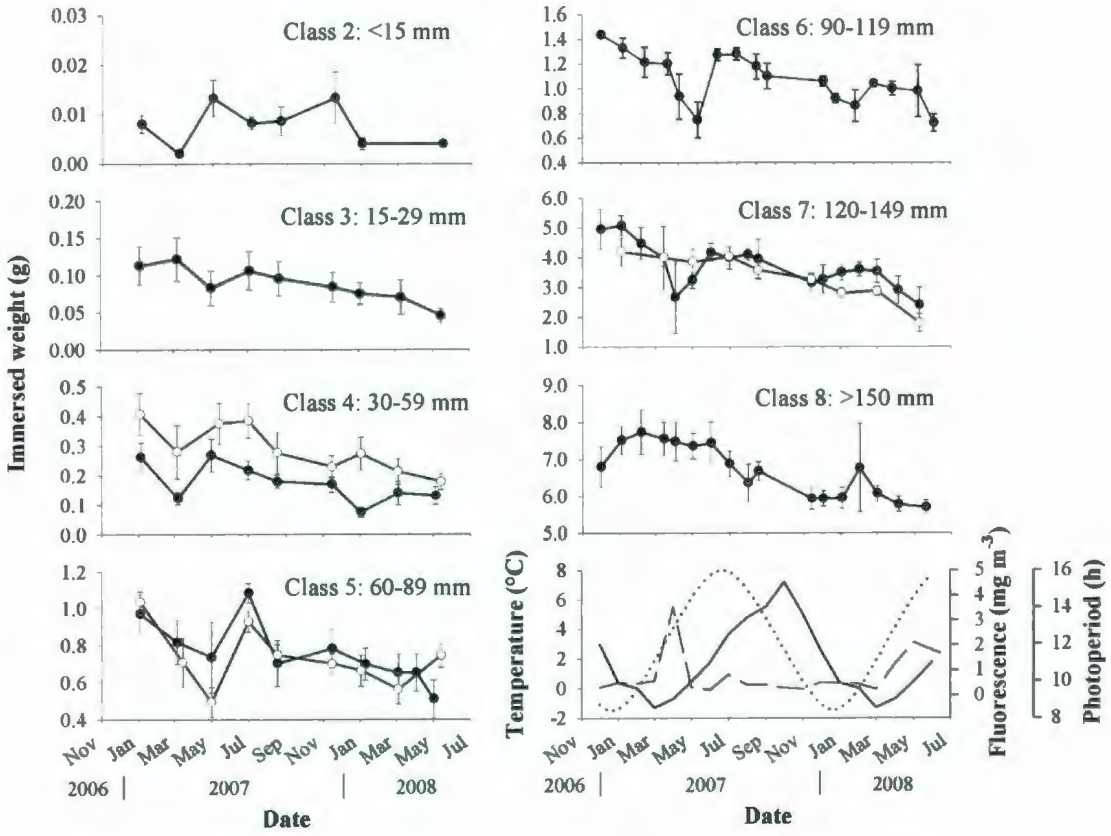

Figure 2-10: Growth cycle from December 2006 to May 2008 using immersed weight of size Classes 2 to 8 of *Cucumaria frondosa* measured under natural environmental conditions including photoperiod (dotted line), temperature (solid line) and chlorophyll a fluorescence (dashed line). Closed circles represent replicate 1 and open circles represent replicate 2, where available. Error bars indicate standard error. See Table 2-2 for size class and replicate descriptions.

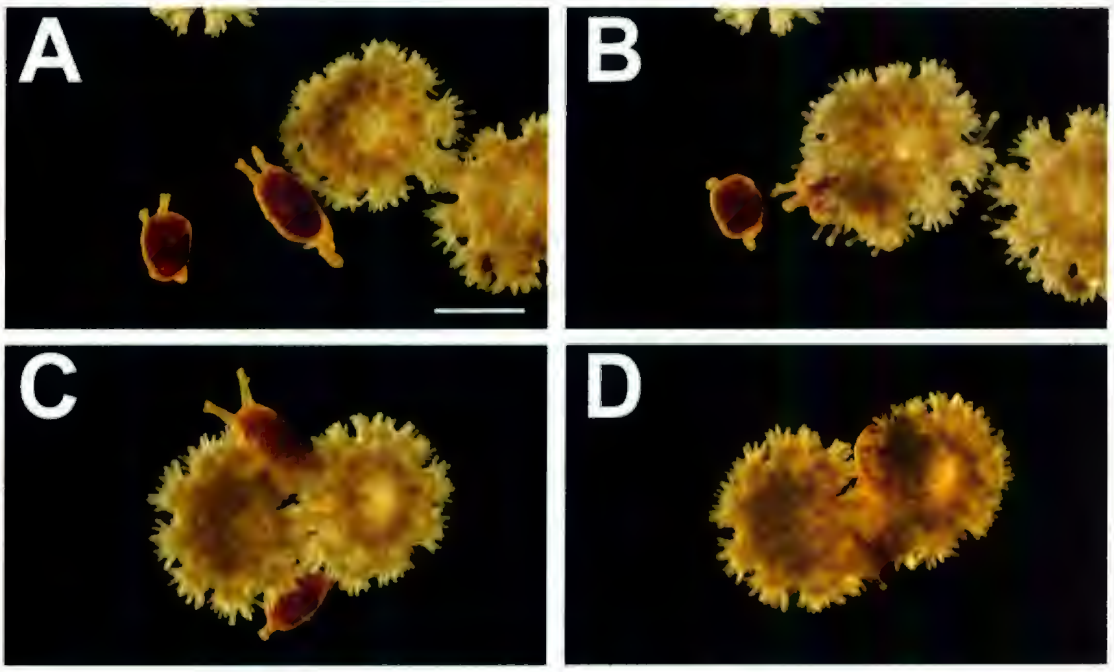

Figure 2-11: Prey-predator interaction between juveniles of *Cucumaria frondosa* and juveniles of *Solaster endeca*. Juvenile sea stars A) moving towards sea cucumbers, B-C) attacking sea cucumbers, D) ready to eat sea cucumbers. Elapsed time is ca. 9 minutes. Scale bar represents 1 mm and applies to all images.

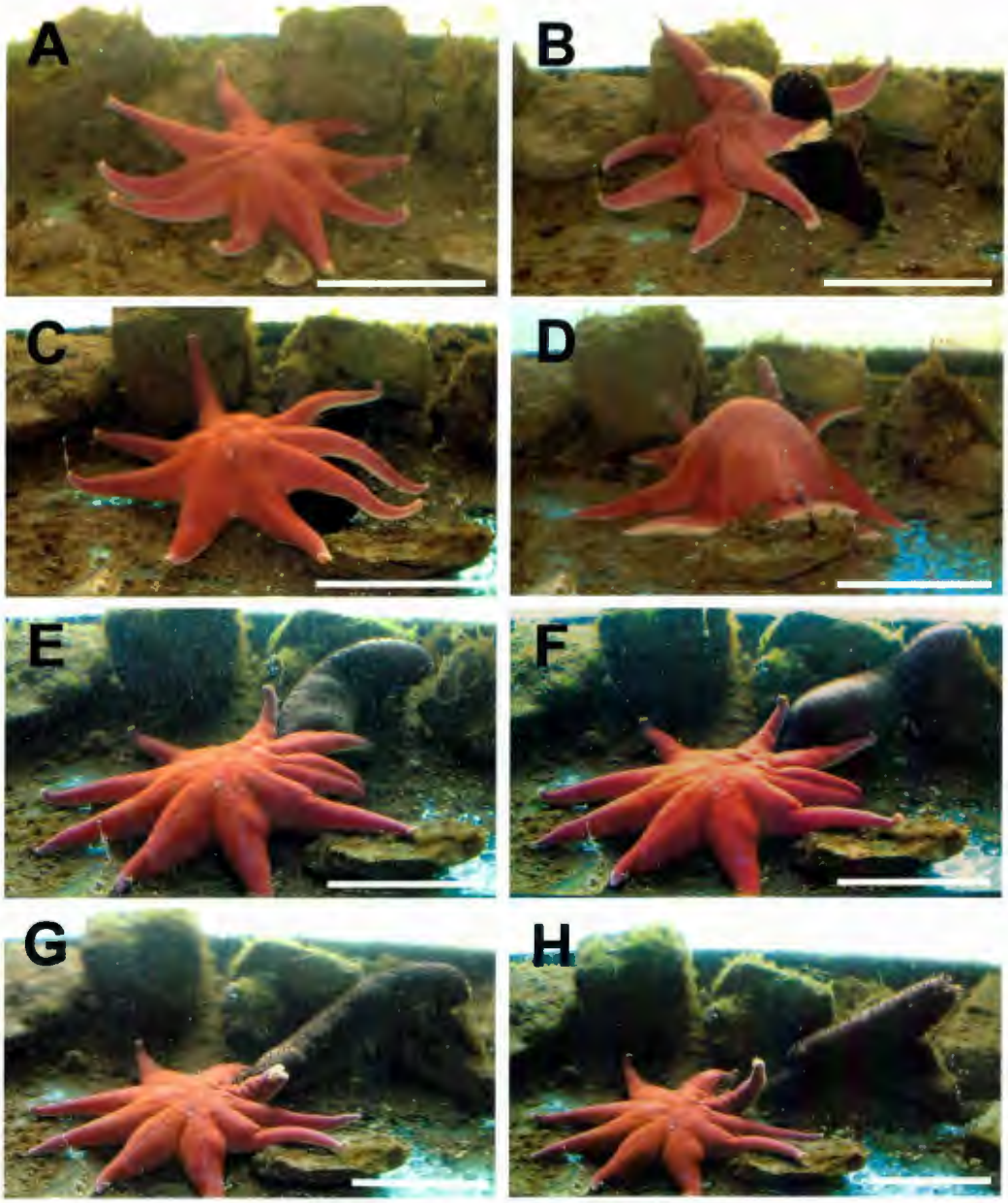

Figure 2-12: Prey-predator interaction between adults of *Cucumaria frondosa* and *Solaster endeca*. The first four images illustrate successful predation by *S. endeca*: A) stationary sea star before introduction of sea cucumber in the tank, B) sea star moving toward sea cucumber and trying to capture it, C) sea star successfully capturing sea cucumber and D) typical position of feeding *S. endeca*. The next four images illustrate successful escape by *C. frondosa*: E) when both species meet, onset of sea cucumber escape response (body bloating), F) sea cucumber enlarging posterior end, pushing away arms of sea star, G) sea cucumber moving away by elongating anterior end towards rocks and H) sea cucumber using rock to attach and contract posterior end to get out of reach. Elapsed time is ca. 40 minutes (A-D) and ca. 2 minutes (E-H).Scale bars represent 10 cm.

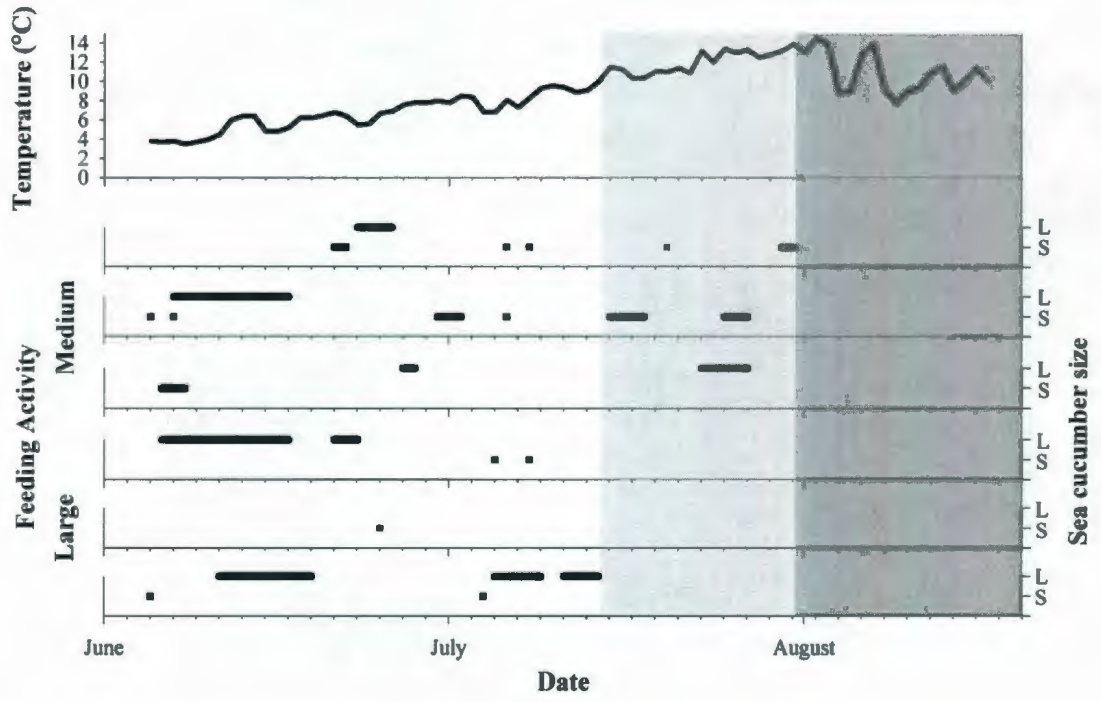

Figure 2-13: Feeding cycle of medium (R: 11.5 ± 0.1 cm; WW: 382.9 ± 40.7 g) and large (14.5 ± 0.5 cm; 621.5 ± 26.0 g) *Solaster endeca*. Sea star feeding on small (CL: 6.4 ± 1.8 cm; IW: 0.6 ± 0.3 g) or large (CL: 14.2 ± 1.8 cm; IW: 5.8 ± 1.1 g) sea cucumbers are indicated by a black line and periods of non-feeding are indicated by gaps. Light grey area shows when large sea stars ceased feeding and dark grey area shows when medium sea stars also ceased feeding.

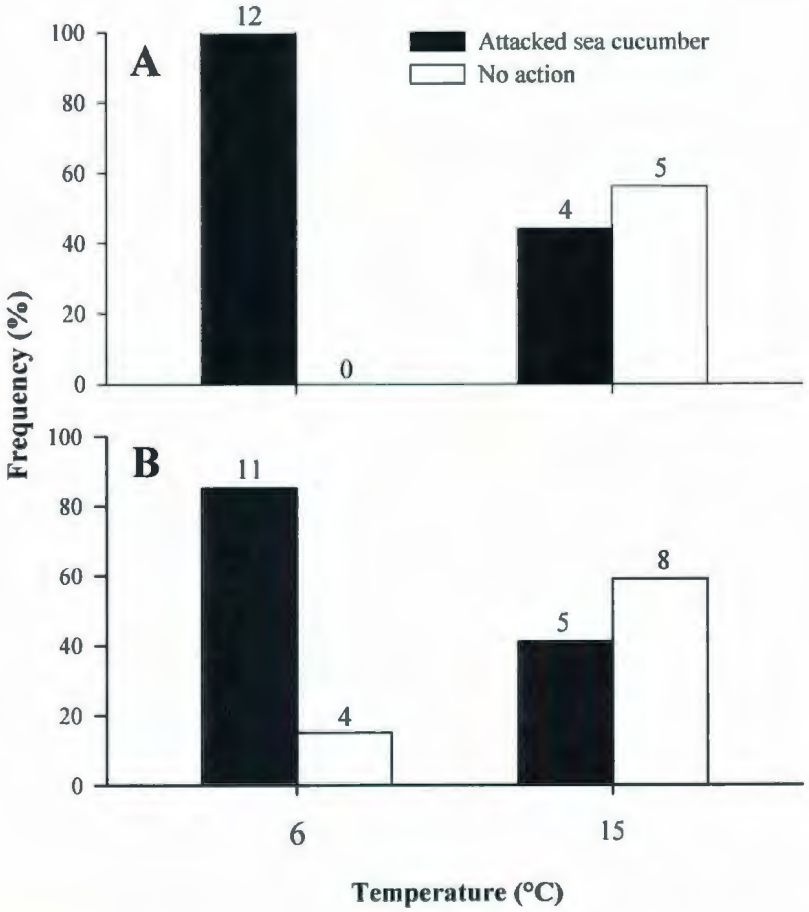

Figure 2-14: Frequency of attacks of A) medium (R: 10.5 ± 1.5 cm), and B) large (14.6 ± 1.5 cm) *Solaster endeca* on *Cucumaria frondosa* at low (6.2 ± 0.3 °C) and high (14.7 ± 0.4 °C) temperatures. Total number of sea stars exhibiting behaviours is noted above each bar.

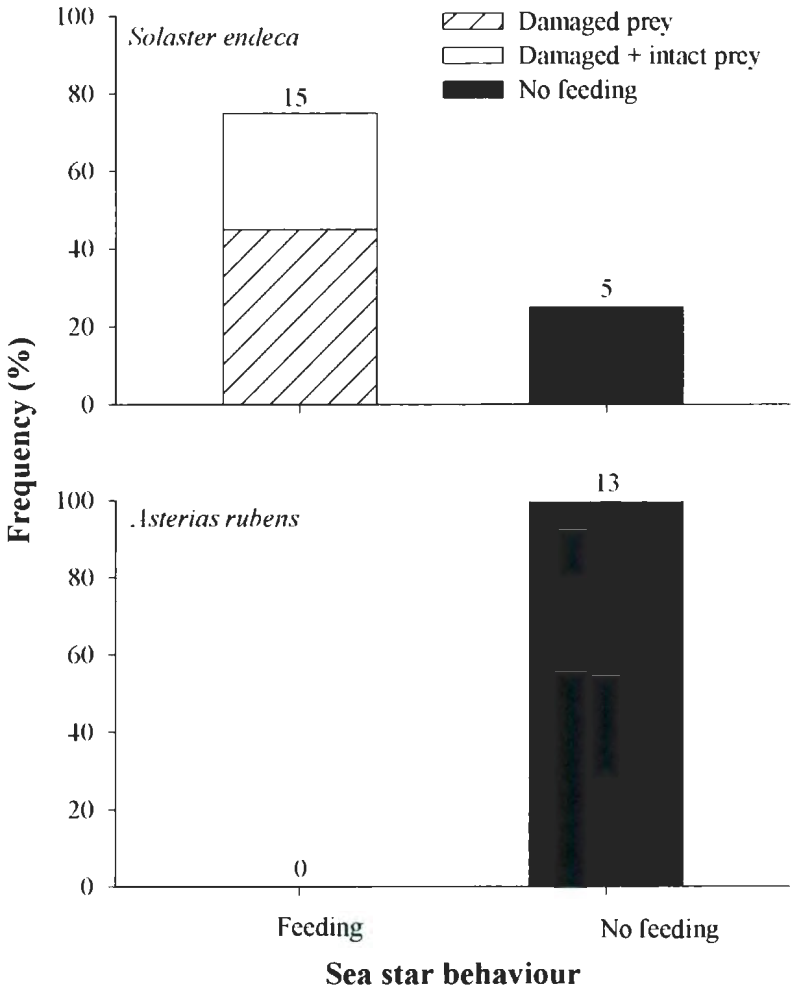

Figure 2-15: Preference expressed by the sea stars *Solaster endeca* and *Asterias rubens* for intact or damaged specimens of *Cucumaria frondosa*. Numbers of feeding and non-feeding individuals are noted at the top of each bar. The white bar indicates feeding first on damaged prey and moving on to feed on intact prey. Feeding first on intact prey or solely feeding on intact prey was not observed.

CHAPTER 3: TRANS-ATLANTIC GLACIAL PERSISTENCE: RECONCILING GENETIC PATTERNS WITH LIFE HISTORY TRAITS IN *CUCUMARIA FRONDOSA* (ECHINODERMATA: HOLOTHUROIDEA)

Spawning of a sea cucumber Cucumaria frondosa *(albino specimen)*

3.1 Abstract

Western North Atlantic marine invertebrate populations were vastly impacted by glaciations during the Last Glacial Maximum (LGM) in the Late Pleistocene. Their patterns of genetic diversity and structure generally indicate extirpation of populations after the LGM and high gene flow across the North Atlantic. *Cucumaria frondosa*, the most abundant and widely distributed sea cucumber in the North Atlantic, was used as a model to test whether its population genetic structure reflects a history of persistence or extirpation and recolonization with respect to glaciations. Mitochondrial DNA (mtDNA) sequences were obtained and analyzed for *C. frondosa* from 20 locations (separated from each other by 7-5900 km) covering most of its distribution range in the North Atlantic. Bayesian skyline plots showed no reduction in effective population size across the North Atlantic during the LGM indicating minimal to no effects by the most recent glacial maximum on populations. Long larval times (ca. 4-7 weeks) and the ability to colonize deep areas to 1300 m may have allowed survival of glaciated areas supporting high haplotype diversity and long population history in the North Atlantic.

3.2 Introduction

Historical environmental conditions that influence phylogeographic patterns of taxa result in different levels of genetic diversity. Such genetic "signatures" in combination with the species biology can be used to determine how populations responded to historical events.

There has been an increasing use of molecular data to infer current and historical phylogeographic patterns of marine invertebrates (e.g. Williams and Benzie 1998; Brown et al. 2001; Duran et al. 2004; Addison and Hart 2004). Genetic drift and mutation occur in populations and create increased population differentiation. This can be counteracted by larval dispersal and other types of gene flow that reduce genetic variation between populations (Slatkin 1987). Due to the contiguous nature of the marine environment, marine invertebrates with the capacity for long distance larval dispersal are generally expected to have little population structure compared to species with non-pelagic larvae (Hellberg 1996; Arndt and Smith 1998; Bohonak 1999). Although many species fit this trend, studies of population structure in the marine environment have shown a surprising amount of genetic differentiation (Benzie 1999; Kyle and Boulding 2000). Genetic breaks have been found in populations in physically contiguous areas but divided by environmental barriers (Reeb and Avise 1990; Williams and Benzie 1998; Sköld et al. 2003). Differences in patterns of population structure have also been observed between species with similar larval dispersal and life history characteristics (Marko 2004). Such discrepencies may occur because recruitment history, natural selection and population turnover can also influence genetic structure (Watts et al. 1990). Differences between

allopatric populations can also arise when they are impacted by environmental and biological pressures to varying degrees.

The Last Glacial Maximum (LGM) of the Late Pleistocene (ca. 18,000 years ago) had major effects on terrestrial and coastal marine habitats in the North Atlantic, displacing and extirpating species through changing environmental conditions and ice scour (Ingolfsson 1992; Maggs et al. 2008). Glacial sheets reaching depths of 400 m spread as far south as New England in the western North Atlantic, but had less severe impacts in the eastern North Atlantic, only reaching as far south as the British Isles (Ingolfsson 1992; Clark and Mix 2002; Dyke et al. 2002). Invertebrates that lived in shallow rocky habitats were particularly impacted as there were few areas of hard substrate beyond the southern glacial limit in the western Atlantic to which they could retreat (Knott and Hoskins 1968). Previous studies have shown that many invertebrates were likely extirpated from the western Atlantic by glaciation and that the area was subsequently recolonized from populations of the eastern Atlantic persisting in glacial refugia south of the glacial limit (Ingolfsson 1992; Wares 2001; Wares and Cunningham 2001; Vermeij 2005). Distribution of species indicates that the majority of marine species from the North American coast are a subset of species from Iceland, which are a subset of species from Europe, suggesting an east to west colonization pattern (Ingolfsson 1992).

The response of marine populations to glacial climate is dependent on their ecology and life history strategy (Hickerson and Cunningham 2005, Wilson 2006; Maggs et al. 2008). Mitochondrial DNA (mtDNA) evidence suggests that some invertebrates species with highly dispersive larval stages were able to survive in glacial refugia in the western Atlantic by colonizing new areas as they became available (Brown et al. 2001;

Wares and Cunningham 2001; Riginos and Henzler 2008). In addition to high dispersal, Hickerson and Cunningham (2006) suggested that ecological plasticity would be essential for survival in glacial refugia as these environments are constantly changing in relation to advancing and receding glaciers.

The sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) is an ideal model to test the hypothesis that certain species survived the LGM. This species mainly inhabits rocky habitats from the lower intertidal zone to depths of ca. 300 m (Klugh 1923; Coady 1973; Gosner 1978; Hamel and Mercier, 1996a). It is the most common sea cucumber in the North Atlantic and one of the most abundant worldwide, reaching densities of 5-50 individuals m^{-2} over vast portions of the sea floor (Hamel and Mercier 1996a; Singh et al. 2001) though its distribution can be patchy in some areas (Therkildsen and Petersen 2006). The lifespan of *C. frondosa* is unknown, but growth to full size (ca. 30 cm) has been estimated to take at least 10 years (Hamel and Mercier 1996a), and possibly as much as 25 years (Chapter 2). As suspension feeders (Singh et al. 1998; Hamel and Mercier 1998) and prey to several other species (Legault and Himmelman 1993; Francour 1997), adults of *C. frondosa* form a key component of many cold-temperate ecosystems. They undergo sexual reproduction annually through broadcast spawning in spring following the phytoplankton bloom and rapid increase in seawater temperature (Hamel and Mercier 1995, 1996b). The buoyant embryos develop into lecithotrophic (non-feeding) larvae that can spend ca. 6 weeks in the water column, regardless of food levels, prior to settlement (Hamel and Mercier 1996a). Most data gathered to date suggest that larval connectivity declines from species with planktotrophic larvae to those with lecithotrophic larvae and is smallest in brooders (Bohonak 1999;

Foggo et al. 2007). The long pelagic lecithotrophic phase in *C. frondosa* suggests potential for dispersal in ranges similar to or higher than most planktotrophs.

Population structure in echinoderms has been studied with respect to contemporary and historical gene flow, mode of larval dispersal, and speciation. Lessios et al. (2001) examined the population structure of sea urchins, *Diadema* spp., in relation to environmental barriers in the southern hemisphere and similar population studies were conducted on the sea star, *Acanthaster planci,* in the South Pacific (Nishida and Lucas 1988; Benzie 1999). In the same area, several population genetic studies were conducted on sea cucumbers in relation to geographic barriers and reproductive modes (Uthicke et al. 1998, 1999; Uthicke and Benzie 2001, 2003). Investigations of gene flow in the genus *Cucumaria* are limited to comparisons between *C. miniata* and *C. pseudocurata* which have lecithotrophic pelagic and brooded larval stages respectively (Arndt and Smith 1998). Mitochondrial DNA indicated that *C. pseudocurata* populations were highly structured, whereas *C. minata*, with larval stages lasting less than two weeks showed no population differentiation on the entire west coast of Canada (Arndt and Smith 1998). Previous molecular studies of *C. frondosa* have been restricted to using mtDNA to identify larvae and assess larval dispersal (Medeiros-Bergen et al. 1995).

To determine the effects of historical glaciations and historical gene flow on populations of *C. frondosa* in the North Atlantic, we sampled individuals through most of the distribution range and analyzed their mtDNA cytochrome *c* oxidase subunit I gene. We interpreted the genetic results in combination with life history characteristics of *C. frondosa* to test the hypothesis that populations were minimally affected in the LGM due to their dispersive larval stages and ecological plasticity.

3.3 Materials and Methods

DNA extraction

A total of 334 sea cucumbers (25-850 g whole animal wet weight) were collected from 20 sample sites between August 2006 and March 2008 by fishermen, researchers and divers (Figure 3-1). Samples were sent to the Ocean Sciences Centre either live, frozen or dissected with tissues preserved in ethanol (EtOH). Mitochondrial DNA was extracted from 5 g of gonad tissue using the Chelex protocol outlined by Medeiros-Bergen et al. (1995). Extracted DNA samples were stored at 4 °C.

Polymerase chain reaction (PCR) amplification and sequencing

Extracted mtDNA was amplified and sequenced using a modified protocol outlined by Uthicke and Benzie (2003). A section of the mitochondrial cytochrome oxidase I (COI) gene (674 nucleotides) corresponding to positions 6001-6674 (from 5'-3') of the mitochondrial genome of the sea urchin *Strongylocentrotus purpuratus* was amplified using the general echinoderm primers CO1e-F – 5'-ATAATGATAGGAGGRTTTGG-3', and CO1e-R – 5'-GCTCGTGTRTCTACRTCCAT-3' (Jacobs et al. 1988; Arndt et al. 1996). The DNA was amplified in 50 µl reactions with the following reagent mix: 2.5 units of Hotstar *Taq* polymerase, 18.5 µl of H_2O, $MgCl_2$ (2.5 µM), PCR Buffer (10x), dNTP mixture (2.0 µM), forward and reverse primers (1 µM) and 5 µl of DNA. PCRs were conducted on thermocyclers (Eppendorf - Mastercycler), with samples loaded at 95 °C and held for 15 min to initialize the Hotstart *Taq* polymerase. This was followed by 35 cycles of 60 s

denaturation at 95 °C, 60 s annealing at 50 °C, 60 s extension at 72 °C. The 35 cycles were followed by a final 10 min extension at 72 °C.

Expected PCR product sizes were verified by electrophoresis on 1 % agarose gels (1xTAE). Montage PCR96 cleanup kit (Millipore) was used to purify products and 20-50 ng ul^{-1} of each purified product was used in subsequent forward and reverse sequencing reactions. For sequencing reactions, Big Dye TM Terminator Cycle Sequencing Kits and AmpliTaq (Applied Biosystems Inc.) were used, following supplier instructions. Sequencing reaction products were purified from unincorporated terminators with ethanol precipitation and electrophoresed on an Abl Prism 3730x1 Analyzer. PCR and sequencing reactions were conducted on Peltier Themal Cyclers (PTC-100 and PTC-225 MJ Research). Forward and reverse sequences were aligned and then edited with Sequencher 4.7 (Gene Codes Corporation) creating consensus sequences for each individual. The mtDNA sequences were submitted to GenBank with the following accession numbers: EU811768-EU8I1824, FJ234231-FJ234400, FJ603323-FJ603432.

Genetic diversity and phylogenetic analysis

Initial Bayesian phylogeneitc analysis conducted in MrBayes (10,000 generations; Ronquist and Huelsenbeck 2003) resulted in average posteriors of 7.56 for the Ti/Tv ratio and of 0.128 for the gamma shape parameter and these estimates were used in subsequent population genetic analysis in Arlequin. Genetic diversity measures and population parameters were estimated with Arlequin version 3.11 (Excoffier et al. 2005). Individual populations were assessed for haplotype and nucleotide diversity (Nei 1987). A haplotype network was constructed using statistical parsimony (Templeton et al. 1992) through the

program TCS 1.21 (Clement et al. 2000) to determine the relationships between haplotypes.

Population expansion and migration rates

Historical population dynamics were further estimated through Bayesian skyline plots approach established by Drummond et al. (2005) implemented by the program BEAST 1.4.8 and visualized in Tracer 1.4.1. Using a set of genetic sequences, BEAST uses Markov chain Monte Carlo (MCMC) sampling procedures to estimate effective population sizes over time. Mutation rates were used to convert time (sub/site/myr) to years. In the absence of specific mutation rates calculated for sea cucumbers, previously calculated sea urchin COI rates of 1.6-3.5 % Myr^{-1} were used (Lessios et al. 1999, 2001; McCartney et al. 2000).

The program MIGRATE 3.0 (Beerli and Felsenstein 2001; Beerli 2008), which uses MCMC maximum likelihood approach to estimate past migration rates between populations was used to estimate rates between the following pooled regions: western, middle and eastern Atlantic. Use of pooled regions avoids the large computational times required to determine rates between individual sample sites. Default settings were used with a Ti/Tv ratio of 7.56.

Population structure

Pairwise F_{ST} genetic distances were calculated between populations and their significance was determined through performing 16,000 permutations in Arlequin, which ensures an exact probability of 99 % (Guo and Thomson 1992). Genetic structure was further analyzed to determine if patterns were reflective of isolation by distance (Nei

1987) by plotting Slatkin's (1993) linearized genetic distance against geographical separation. Correlation and significance were calculated between the two matrices using the Mantel permutation procedure. Hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was conducted to assess genetic structure in the North Atlantic. Analysis of molecular variance partitions total variance of Kimura (1980) two-parameter genetic distances into covariance components with respect to groupings; among populations, among defined regions and within populations. Covariance components were used to calculate Φ-statistics (Excoffier et al. 1992) analogues of Wright's (1951) F-statistics. Calculated Φ-statistics correspond to the correlation between the variance of defined groups compared to all sampled populations (Φ_{CT}), populations within groups compared to the variance of defined groups (Φ_{SC}) and all populations compared to the total variance of all sampled populations (Φ_{ST}). Populations were grouped into western, mid- and eastern Atlantic regions (Table 3-1) for analysis where phylogeographic subdivision has been previously detected in other marine invertebrates (Wares and Cunningham 2001; Addison and Hart 2005). Both pairwise F_{ST} and AMOVA were conducted using Kimura (1980) two-parameter molecular distance of haplotypes into consideration. Population structure was further analyzed with a spatial analysis of shared haplotypes (SAShA); a novel technique to determine if observed distributions of shared haplotypes deviated from expected distribution under panmixa (Kelly et al. in press). This statistical analysis assumes that haplotypes co-occurring in separate locations are identical by descent and are evidence of gene flow. Comparing a random distribution of shared haplotypes to the actual distribution, determines if these are randomly distributed. This statistic is unimformative where sequences have a patchy distribution,

therefore a cumulative distribution function(D_{cdf}) is calculated which accounts for this. This statistic compares the cumulative expected and observed differences across all geographic distances. Unlike F_{ST} and AMOVA, SAShA is able to determine subtle levels of population structure despite gene flow or within population variance (Kelly et al. in press).

Populations sampled within NAFO area 3K (3KC) and in Bergen, Norway (BEN) were removed from all demographic statistical analyses with the exception of diversity statistics and the haplotype network due to small sample size (n=4, Table 3-1). Omission of small sample populations from the analyses did not distinctly alter the outcome of the AMOVA or expansion model.

Laboratory studies

To assess the larval dispersal capabilities of *C. frondosa* adults and juveniles were collected from various areas around Newfoundland and maintained in tanks for over two years. They were kept under natural photoperiod within a flow-through seawater system maintained at ambient temperatures (-1-15 °C) that provided natural phytoplankton levels. In 2006 and 2007, the sea cucumbers spawned in several of the tanks. The floating eggs were collected, checked for an elevated fertilization coat and transferred into culture vessels provided with running seawater and rock substrate. The developing larvae were observed regularly to determine time to settlement and monitor subsequent growth. Other biological and behavioral observations were made throughout this study (see Chapter 2 for details).

3.4 Results

Sequences of 618 bp length were obtained from 334 specimens of *C. frondosa* yielding 143 separate haplotypes (Table 3-1).

Genetic diversity and phylogenetic analysis

All sample sites showed high genetic variability with haplotype diversity ranging from 0.849 to 1.000 (Table 3-1). Haplotype diversity for pooled populations in the western and eastern Atlantic was 0.955 and 0.950 respectively. Nucleotide diversity ranged from 0.0045 to 0.0085 across the North Atlantic. The number of haplotypes per site ranged from 4 to 24 with no obvious patterns of distribution in relation to geographical location. The most common haplotype (seq J) was found in 17 of 18 major sample sites. Private haplotypes comprised 33 % (n=109) of all haplotypes and ranged from 0-14 haplotypes per sample site. Site-specific haplotypes occurred at low frequencies but comprised 86 % of all haplotypes found.

Maximum parsimony resulted in a single haplotype network (Figure 3-2) that did not reveal any partitioning across the North Atlantic with most internal haplotypes being shared across all regions or between the western Atlantic and eastern or mid-Atlantic regions. However, the haplotype, located centrally within the network (N; 10 individuals) with the most connections, was found in the western and mid-Atlantic. In contrast, all haplotypes unique to the eastern Atlantic are tip haplotypes.

Population expansion and migration rates

F_S values were significantly negative ($p<0.050$; 1000 random samples) for individual populations and across regions (Table 3-1). Bayesian skyline plot (Figure 3-3) showed increasing effective population size for the entire North Atlantic over the past 71 000-156 000 years. A large increase in effective population size occurred 14 300-31 300 years before present (ybp). In the Western Atlantic, two major effective population increases occurred 20 000-44 000 and 51 000-113 000 ybp. The mid and Eastern Atlantic populations have been continually increasing in size with no large increases. Time to most recent common ancestor occurred 127 000-277 000 ybp in the Western Atlantic, 126 000-275 000 ybp in the mid-Atlantic and 119 000-260 000 ybp in the Eastern Atlantic.

Effective migration rates were high across North Atlantic regions, with asymmetric gene flow between the mid- and western Atlantic and comparable exchange between western and eastern Atlantic (Table 3-2). Asymmetric rates were also detected between mid- and eastern Atlantic with gene flow oriented in a west to east direction.

Population structure

Pairwise corrected Kimura two-parameter F_{ST} values were generally low with few significant values (Table 3-3). Cook's Harbour, Canada (CHC) and Møerkvedbukta, Norway (MON) were significantly ($p<0.05$) differentiated from several sites. Linearized genetic distance plotted against geographic distance showed weak increasing population structure with increasing distance (Mantel's test $r=0.32$, $p=0.028$; Figure 3-4).

Analysis of molecular variance indicated that 98 % of the variation detected was within populations (Φ_{ST}=0.018; p=0.031; Table 3-4). Significant variation was detected with AMOVA between the eastern and western regions of the North Atlantic (Φ_{CT}=0.016; p=0.047), but not between any other regions. Small but significant variation was also detected among populations within the Eastern and Western Atlantic ((Φ_{ST}=0.024; p=0.041) A total of 13 haplotypes were shared between the eastern and western Atlantic.

Examination of spatial analysis of shared haplotypes (SAShA) indicated that the mean distribution of shared haplotypes was not significantly different from distribution expected under panmixia (p=0.534; Figure 3-5A). The cumulative frequency distribution statistic showed similar results indicating non-significant deviations from expected panmictic distribution (D_{cdf}=0.047; p=0.621; Figure 3-5B).

Laboratory studies

Sea cucumber larvae settled after reaching the pentactula stage indicated by the development of five oral tentacles and two ambulacral podia as well as the concomitant loss of buoyancy (Hamel and Mercier 1996a; Chapter 2). Settlement under laboratory conditions started ca. 42 days after spawning. Table 3-5 summarizes biological data compiled in this and previously published studies that are relevant to glacial persistence.

3.5 Discussion

The analysis of mtDNA of *C. frondosa* populations reveals persistence through glaciations of the Late Pleistocene across the North Atlantic and high historical gene flow

consistent with the biology of the species. The results of this study are different from the phylogeography determined in many previously studied marine invertebrates and may provide insight into ecological characteristics needed to survive glacial conditions.

Glacial persistence in the North Atlantic

Populations of species that have survived glacial periods are expected to have a high haplotype diversity because under a neutral model diversity is expected to be higher in populations with longer histories than in recently founded populations (Wares and Cunningham 2001; Maggs et al. 2008). Wares and Cunningham (2001) found that several obligate rocky intertidal invertebrates supported Ingolfsson's (1992) view of extirpation in the western North Atlantic followed by an east to west recolonization, based on lower haplotype diversities in the western than the eastern regions. Our results indicate a high diversity in most areas sampled with no significant differences between the eastern and western Atlantic. However differences may be masked by uneven sampling and high within population variation. Sampled areas for the eastern Atlantic were only available from western Norway which was ice covered during the LGM (Clark and Mix 2002) indicating that this area was likely recolonized by populations in southern refugial areas (Maggs et al. 2008). In the case of an east to west recolonization, Norway would likely still have been recolonized at a faster rate than the western Atlantic and higher genetic diversity would be expected. We agree that an east to west recolonization is plausible for many obligate rocky shallow-water invertebrates (Wares and Cunningham 2001); however the high haplotype diversity observed in *C. frondosa* supports the hypothesis of persistence through the LGM. Maggs et al. (2008) suggested that high haplotype diversity

may be an indicator of areas of secondary contact between separated refugial populations, however diversity was consistently high across the North Atlantic. In addition the range of *C. frondosa* has shifted since the LGM and no longer extends to supposed refugial areas in the eastern Atlantic

Survival of *C. frondosa* populations through the most recent glaciations is further evidenced by estimated demographic history which shows no reduction in effective population size. In the western Atlantic, large population increases are observed soon after the LGM (ca. 18 000 years ago). Although glaciations may not have had any negative effects on populations, it may have reduced areas to which *C. frondosa* populations could have expanded. Thus, recession of glaciers, may have allowed spatial and demographic expansion of populations. In the eastern and mid-Atlantic regions, population increases were minimal indicating low demographic expansion in those areas. Glaciations were not as severe in the mid to eastern Atlantic (Clark and Mix 2002) suggesting that populations may have already colonized suitable areas and were not vastly affected by the LGM. Subsequent expansion after the LGM is also supported by negative neutrality values (Tajima 1989; Fu 1997). Selective pressures would also create this pattern but would also reduce haplotype diversity which is not observed in our results.

Time to most recent common ancestor was estimated to occur between 119 000 and 277 000 years ago across the North Atlantic coinciding with glacial maxima followed by interglacial periods indicating demographic expansion. Glacial conditions were more severe and ice sheets were thicker during previous glacial periods than during the LGM (Clark et al. 1999) and a similar timeline of extinction and bottlenecks has been observed in bivalve populations (Stanley 1986).

Persistence through the Pleistocene glaciations in separate refugia is often reflected by reciprocal monophyly (Cunningham and Collins 1998; Hickerson and Cunningham 2006) but this was not evident for *C. frondosa* which may be the result of ongoing gene flow. Although small amounts of differentiation were detected between the eastern and western Atlantic, there is no indication that there was limited connectivity between the two regions. Populations of *C. frondosa* living on the eastern Grand Banks would have been open to larval exchange with populations in the eastern Atlantic, specifically Iceland and southern Norway. Furthermore, lower sea level during the LGM would have created habitable areas that are currently unavailable. The high number of shared haplotypes between eastern and western North Atlantic is further evidence for uninterrupted connectivity irrespective of mid-Atlantic populations. A large founding population could also explain this trend, supported by historical currents (Vermeij 1991), however, evidence of long population histories and a large number of descendent haplotypes allow us to reject this scenario.

To date, trends of persistence through glacial conditions in eastern Canada supported by molecular and paleontological data have been detected in two fish, the rock gunnel, *Pholis gunnellus* (Hickerson and Cunningham 2006) and Atlantic cod *Gadus morhua* (Bigg et al. 2008) and several planktotrophic marine invertebrates, including the sea star, *Asterias forbesi* (Wares 2001), the green sea urchin *Strongylocentrotus droebachiensis* (Addison and Hart 2005), the rock barnacle *Semibalanus balanoides* (Brown et al. 2001), clams in the *Macoma bathica* complex (Nikula et al. 2007) and the blue mussel *Mytilus edulis* (Riginos and Henzler 2008). This is the first time that glacial persistence is evidenced in a member of the class Holothuroidea and in a lecithotrophic

marine invertebrate in the North Atlantic. The high contemporary biomasses of *C. frondosa* measured in certain areas of the western North Atlantic (up to ca. 300,000 tonnes; Hamel and Mercier 2008) are consistent with long-established populations.

Constant expansion of effective population size is consistent with the haplotype network, which shows each of the major and minor haplotypes surrounded by descended rare alleles, creating a star shaped pattern reflective of demographic expansions (Slatkin and Hudson 1991). This pattern, which has been observed in other echinoderms (Uthicke and Benzie 2003; Duran et al. 2004), and some crustaceans (Stamatis et al. 2004), is indicative of an enormous population size which is able to retain new mutations during rapid population growth or expansion (Avise et al. 1984; Watterson 1984). This trend has been associated with seawater level changes, which increase and decrease available habitat (Fauvelot et al. 2003). In the North Atlantic, the combination of dynamic changes in glacial cover and sea-level are likely the cause of this pattern in *C. frondosa* (Clark et al. 1999; Maggs et al. 2008).

Following coalescent theory (Crandall and Templeton 1993) more frequent haplotypes with many connections are phylogenetically older than low frequency tip haplotypes. Therefore, the fact that central haplotypes are shared across regions of the North Atlantic regardless of sampling effort provides some evidence for high gene flow from the eastern Atlantic.

Population structure

The population structure of *C. frondosa* suggests that there has been high gene flow across the North Atlantic with tendencies towards panmixis, and some local

heterogeneity. Nonetheless, AMOVA suggested some restrictions in gene flow between major regions. Corrected pairwise genetic distances were largely non-significant suggesting little population differentiation. Some genetic patchiness was observed however as several pairwise differences between populations were statistically significant, although not highly so. Similar patterns of low structure have been attributed to high gene flow and were observed in other holothuroids with shorter planktonic larval times (Uthicke and Benzie 2003; Arndt and Smith 1998). These results were consistent with the distribution of shared haplotypes that was similar to a distribution under panmixia. The patchy distribution found in populations of *C. frondosa* can mask differences between simulated and observed haplotype distribution, however the cumulative differences were also not significantly different from expected panmictic results (Kelly et al. in press). This novel analysis is ideal for species such as *C. frondosa* with high migration rates which tend to reduce the effectiveness of Φ_{ST} to detect population structure. Unlike AMOVA, SAShA provides a more robust analysis that is able to determine subtle levels of genetic structure without biases from *a priori* grouping. Panmictic patterns of population structure also support high historical gene flow due to expansion from refugium.

Patterns of local heterogeneity with global tendency towards panmixis have been observed in the sea urchin *Paracentrotus lividus* in the North East Atlantic (Duran et al. 2004), the limpet *Siphonaria* sp. in the South Pacific (Johnson and Black 1982) and the gastropod *Colleisella subrugosa* in the South East Atlantic (José and Solferini 2007). Variations in history of recruitment, genetic composition of recruits, population turnover

and post-settlement selection are all factors that could cause genetic patchiness (Watts et al. 1990).

Mid-Atlantic region

In the present study Icelandic samples from the mid-Atlantic were considered separate from western and eastern Atlantic samples. Previous studies have grouped Icelandic populations with the eastern Atlantic as it may have provided a stepping stone for recolonization of the North American coast (Ingolfsson 1992; Wares and Cunningham 2001). Grouping of mid-Atlantic samples with eastern and western populations can be difficult as various species within the same area show differences in affinity for either region (Rignos and Henzler 2008 and references therin). Molecular studies by Reeb and Avise (1990) and Riginos and Henzler (2008) found more similarity in bivalve populations between North America and Iceland than between Europe and Iceland. The opposite trend has been observed in fish (Hickerson and Cunningham 2006; Bigg et al. 2008) and sea urchins (Addison and Hart 2005). Populations of *C. frondosa* from western Iceland show higher affinity for North America (western) than Norway (eastern) populations as they share a root haplotype in the middle of the haplotype network. Western Atlantic populations of *C. frondosa* share the same number of haplotypes with Iceland and with Norway; however no shared haplotypes were found only between Iceland and Norway. This pattern may be explained by effective migration rates between the three regions and is likely due to particularities of North Atlantic currents that link these areas together (Figure 3-1; Fratantoni 2001). Analysis of gene flow rates shows higher exchange of effective migrants between North America and Iceland than between

North America and Norway (Table 3-2). North Atlantic circulation moves in a counter clockwise direction with current velocities highest in the western Atlantic and slowest in the eastern Atlantic (Fratantoni 2001; Flatau et al. 2003). The North Atlantic Current splits into two main branches; the first heading north east towards Norway and the other moving westerly towards Iceland. In the opposite direction, the Icelandic and Western Greenland Currents likely carry migrants towards North America. The direction of the Norwegian Current supports restricted larval exchange between Norway and Iceland. A surprisingly high gene flow was observed between Norway and North America which accounts for the high number of shared haplotypes between the regions. Although the North Atlantic Current would account for migrants from North America, the reasons for gene flow in reversed direction is unclear. Further exploration of trans-arctic gene flow is needed to determine if it is a possible pathway of exchange to *C. frondosa*. The disparate patterns in gene flow also highlight the sporadic nature of larval dispersal between regions.

Ecology and larval dispersal

If *C. frondosa* persisted in the western North Atlantic through the last glacial maximum where many other marine taxa have not (Wares and Cunningham 2001), what factors enabled it to survive changing glacial conditions? Although dispersal ability has been noted as a main determinant (Wares and Cunningham 2001), the biology and ecology of a species are key factors in survival as evidenced by the different demographic histories *A. rubens* and *A. forbesi*, during and postdating the LGM (Wares 2001). *Cucumaria frondosa* is an ecologically flexible Atlantic and Arctic species, capable of

living to depths >300 m and of tolerating very cold temperatures (Smirnov 1994). Its larval development is not impaired by low salinities (Hamel and Mercier 1996a) and adults are known to occur in estuaries (Hamel and Mercier 1996a; Singh et al. 2001). Hence, populations were likely able to persist close to the glacial limits, enabling them to colonize areas quickly as glaciers receded. Colder ocean temperatures would also extend larval development times (Hamel and Mercier 1996a) therefore prolonging the pelagic phase and favoring dispersal toward new areas. Sea cucumbers such as *C. frondosa* also demonstrate gonadal plasticity, and the capacity to develop cohorts of gametes over multiple years to sustain annual spawning in colder regions (Hamel and Mercier 1996b). Moreover, adults are able to maintain gametogenesis and spawning through prolonged starvation periods (Hamel and Mercier 1996c). Although *C. frondosa* generally colonizes rocky substrates, it has recently been observed on sandy patches over the Grand Banks of Newfoundland (CSAR 2006) suggesting that it can adjust to various habitats. Such environmental plasticity would allow *C. frondosa* to easily adapt to a changing glacial climate and effectively colonize areas unsuitable for other obligate rocky invertebrates. It is even possible that *C. frondosa* was able to find refuge in deep waters as mature individuals have been found down to 1300 m. In addition, deep-water stepping stone populations in the mid-Atlantic would increase connectivity between eastern and western regions. Although it was not possible during this study, gene flow analysis of samples collected over a wide bathymetric gradient would allow us to test this deep-sea refugium hypothesis.

Cucumaria frondosa exhibits high historical gene flow across the North Atlantic and weak isolation by distance consistent with a stepping stone dispersal model. The

direction of historical gene flow follows dominant ocean currents, providing further support that larval dispersal is a main component affecting population structure. This study is in contradiction with the long held paradigm that pelagic lecithotrophic larvae are poorly dispersive compared to planktotrophic larvae (Duran et al 2004). However, it is not the first time this prediction has been questioned (Young et al. 1997) and evidence of high larval dispersal in *C. frondosa* has been reported (Hamel and Mercier 1995; Medeiros-Bergen et al. 1995). Given the larval time we measured (ca. 42 days), *C. frondosa* larvae from the western Atlantic could travel an average of 1500 km in the North Atlantic Current at an average speed of 38 cm s^{-1}. Although this does not span the North Atlantic, it may enable *C. frondosa* to reach areas around the mid-Atlantic ridge or the continental shelf of the eastern Atlantic. In addition, this may be a conservative estimate of larval dispersal as the North Atlantic Current may reach speeds of 209 cm s^{-1} and we did not factor in the potentially longer larval duration in consistently cold water (Hamel and Mercier 1996a).

Single locus phylogeography

This study employs single locus (COI) mtDNA to establish the population history of *C. frondosa* in Atlantic waters. Studies with single locus markers may overestimate population demographic parameters (Brumfield et al 2003), thus multiple unlinked markers would improve statistical estimates. Although eight regions previously amplified in other *Cucumaria* species (Arndt and Smith 1998) were tested (data not shown), only the COI region showed enough variability to elucidate phylogenetic patterns. For future studies a combination of markers which undergo varying rates of mutation and highlight

different aspects of demographic history, should be used to provide a finer resolution of historical population dynamics (Addison and Hart 2005). Nonetheless, this study adds to the growing body of evidence for species-specific persistence through glacial periods and lays the groundwork for further research in the under-investigated area of holothurian molecular phylogeny in the North Atlantic.

Conclusions

The population structure of *C. frondosa* is characterized by large population size, high historical gene flow and high haplotype diversity. There is weak evidence of isolation by distance, but small amounts of genetic structuring at local levels are consistent with their patchy distribution and sporadic gene flow. All these factors contribute to the small genetic variations observed between eastern and western regions. Results from mtDNA suggest that populations of *C. frondosa* persisted through the LGM throughout the North Atlantic. Although larval dispersal likely played an important role in population expansion before and after the LGM, ecological flexibility was presumably even more essential to the survival of *C. frondosa* through glaciations.

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3.8 Tables

Table 3-1: Diversity measures for populations of *Cucumaria frondosa* using 618 bp of COI. The number of samples per site (N), unique haplotypes (H) and polymorphic sites (PS) are listed as well as Fu's F_S neutrality value. Standard deviation is noted in brackets and significant values are shown in bold.

Site ID	Sampling location	N	H	PS	Haplotype diversity	Nucleotide diversity	F_S
	Canada						
BIC	Belcher Island, NU	15	14	18	0.991 (0.028)	0.0060 (0.0036)	**-5.01**
HSC	Hudson Strait, NU	13	9	12	0.872 (0.092)	0.0050 (0.0031)	**-1.37**
WHC	William's Harbour, NL	17	13	18	0.971 (0.028)	0.0063 (0.0030)	**-2.26**
LLC	L'ance au Loup, NL	18	15	21	0.971 (0.039)	0.0072 (0.0041)	**-3.31**
CHC	Cook's Harbour, NL	29	21	22	0.970 (0.018)	0.0070 (0.0040)	**-4.62**
3KC	NAFO Area 3K, NL	4	4	5	1.000 (0.176)	0.0045 (0.0035)	**-0.34**
TWC	Twillingate, NL	17	15	25	0.978 (0.031)	0.0078 (0.0045)	**-3.28**
HBC	Hare Bay, NL	25	24	33	1.000 (0.027)	0.0085 (0.0048)	**-10.41**
ACC	Admiral's Cove, NL	15	13	19	0.981 (0.030)	0.0057 (0.0034)	**-2.44**
SPC	St. Pierre Bank, NL	9	6	13	0.889 (0.091)	0.0064 (0.0040)	1.99
4WC	NAFO Area 4W, NS	14	11	20	0.934 (0.061)	0.0082 (0.0047)	**-0.40**
HPC	Havre-Saint-Pierre, QC	8	6	11	0.928 (0.084)	0.0067 (0.0043)	0.27
P1C	Passamaquoddy Bay (1), NB	21	17	21	0.967 (0.030)	0.0067 (0.0038)	**-4.57**
P2C	Passamaquoddy Bay (2), NB	25	16	25	0.900 (0.053)	0.0061 (0.0035)	**-2.69**
	United States						
CNU	Cape Neddick, ME	18	15	21	0.974 (0.029)	0.0072 (0.0041)	**-3.69**
CBU	Casco Bay, ME	16	12	23	0.959 (0.036)	0.0076 (0.0044)	**-1.12**
	Western Atlantic Total	264	129	101	0.955 (0.008)	0.0018 (0.0091)	**-23.96**
	(Mid-Atlantic) Iceland						
AKI	Akranes	31	21	22	0.957 (0.025)	0.0071 (0.0040)	**-4.73**
	Norway						
SKN	Skaug	12	5	8	0.849 (0.059)	0.0055 (0.0034)	3.95
MON	Mørkvedbukta	23	13	15	0.949 (0.023)	0.0056 (0.0033)	**-1.82**
BEN	Bergen	4	4	7	1.000 (0.176)	0.0083 (0.0060)	**1.00**
	Eastern Atlantic Total	39	19	21	0.950 (0.015)	0.0062 (0.0035)	**-2.02**
	Entire North Atlantic	334	143	110	0.956 (0.007)	0.0059 (0.0033)	**-23.91**

Table 3-2: Effective migration rates of populations of *Cucumaria frondosa* between three regions in the North Atlantic calculated with MIGRATE (Beerli 2008). Upper and lower 95 % profile confidence limits are noted in brackets.

		Migration rates		
	n	**North America**	**Iceland**	**Norway**
North America to	260	-	228 (198-438)	564 (427-892)
Iceland to	31	913 (859-1130)	-	680 (597-1250)
Norway to	35	465 (428-605)	56 (36-892)	-

Table 3-3: Pairwise F_{ST} values of *Cucumaria frondosa* across the North Atlantic calculated with 16002 permutations of haplotypes. Significant values ($p<0.05$) are shown in bold. Refer to Table 3-1 for sample site identification. Sites 3KC and BEN were excluded due to low sample size. Negative values are the result of imprecision in the calculations and can be interpreted as zero (Long 1986).

	Western Atlantic																Eastern Atlantic	
	BIN	HSC	WHC	LLC	CHC	TWC	HBC	ACC	SPC	4WC	HPC	P1C	P2C	CNU	CBU	AKI	SKN	MON
BIN																		
HSC	-0.022																	
WHC	-0.025	-0.018																
LLC	0.003	-0.009	0.015															
CHC	0.024	0.002	0.019	0.017														
TWC	0.005	-0.013	0.016	-0.010	0.055													
HBC	-0.008	-0.007	0.017	-0.007	**0.045**	0.009												
ACC	-0.029	-0.004	0.004	0.018	0.037	0.024	-0.007											
SPC	-0.024	-0.012	0.000	0.004	0.015	0.004	-0.023	-0.046										
4WC	-0.010	-0.019	0.016	-0.021	**0.003**	-0.003	-0.001	0.005	-0.004									
HPC	-0.026	-0.014	-0.001	-0.033	-0.005	0.006	-0.028	-0.018	-0.028	-0.047								
P1C	-0.017	-0.016	-0.005	0.011	0.015	0.026	-0.004	0.000	-0.006	-0.011	-0.026							
P2C	-0.019	-0.003	0.015	0.008	**0.041**	0.010	-0.011	-0.011	-0.026	-0.001	-0.018	-0.012						
CNU	-0.001	0.002	0.021	0.012	0.024	0.029	-0.004	-0.001	-0.021	0.020	-0.025	0.004	-0.007					
CBU	-0.017	-0.001	-0.007	0.019	-0.003	**0.046**	0.019	0.001	0.005	0.001	-0.030	-0.018	0.005	-0.001				
AKI	0.005	-0.003	0.005	0.004	0.005	0.044	0.015	0.021	0.004	0.017	-0.012	-0.006	0.012	-0.005	-0.009			
SKN	-0.002	0.041	0.035	0.011	**0.069**	0.017	0.034	0.050	0.064	-0.001	0.005	0.025	0.026	0.055	0.034	**0.054**		
MON	0.008	0.045	0.012	**0.053**	**0.036**	**0.098**	**0.050**	0.027	0.050	0.035	0.004	0.001	0.036	**0.047**	-0.011	0.011	0.048	

Table 3-4: Hierarchical analysis of molecular variance of populations of *Cucumaria frondosa* across the North Atlantic.

Source of variation	df	Percentage of variation	Fixation indices
All regions	2	1.11	Φ_{CT}=0.011
Within groups	15	0.68	Φ_{SC}=0.006
Within populations	308	98.21	Φ_{ST}=0.018*
Western and mid-Atlantic	1	0.55	Φ_{CT}=0.005
Within groups	14	0.41	Φ_{SC}=0.004
Within populations	275	99.04	Φ_{ST}=0.009
Western and eastern Atlantic	1	1.67	Φ_{CT}=0.018*
Within groups	15	0.72	Φ_{SC}=0.007
Within populations	278	97.60	Φ_{ST}=0.024*
Eastern and mid-Atlantic	1	-0.65	Φ_{CT}=-0.006
Within groups	1	3.62	Φ_{SC}=0.036
Within populations	63	97.03	Φ_{ST}=0.029

*Significant at $p<0.05$ after 10100 permutations

Table 3-5: Ecological characteristics of *Cucumaria frondosa* that support survival through last glacial period and subsequent population expansion.

Traits	Characteristics	Sources
Feeding	Feeds on phytoplankton, phytodetritus and other particulate matter.	This study; Hamel and Mercier 1998
	Seasonal feeding attuned to food abundance; can withstand prolonged fasting periods.	Hamel and Mercier 1998; Singh et al. 1998; 2001
	Fasting for over one year does not prevent reproduction.	Hamel and Mercier 1996c
Reproduction	Iteroparous.	This study, Hamel and Mercier 1995; 1996a
	Massive annual broadcast spawning correlated with environmental factors.	Hamel and Mercier 1995; 1996b; Singh et al. 2001
	High natural fertilization rates.	Hamel and Mercier 1996d
	Gametogenic synchrony between distant populations by chemical communication.	Hamel and Mercier 1996c; 1999
	Intermediate fecundity (ca. 9,000 eggs).	Hamel and Mercier 1996a
Larval development and ecology	Naturally long larval development times (ca. 4-7 weeks).	This study; Hamel and Mercier 1996a
	Prolonged larval time under colder temperatures.	Hamel and Mercier 1996a
	Larvae can withstand low salinities.	Hamel and Mercier 1996a
	Positively buoyant larvae found high in water column.	Medeiros-Bergen et al. 1995; Hamel and Mercier 1996a; 1996b
Population ecology	Can colonize great depths (ca. 1300 m).	J.-F. Hamel, personal observation
	Can colonize bedrock, boulder, gravely and sandy habitats.	CSAR 2006; Klugh 1923
	Can be found in areas of intermediate salinity (e.g. estuaries).	Hamel and Mercier 1996a; Singh et al. 2001
	Adapted to Arctic conditions.	Smirnov 1994; Gudimova et al. 2004

3.9 Figures

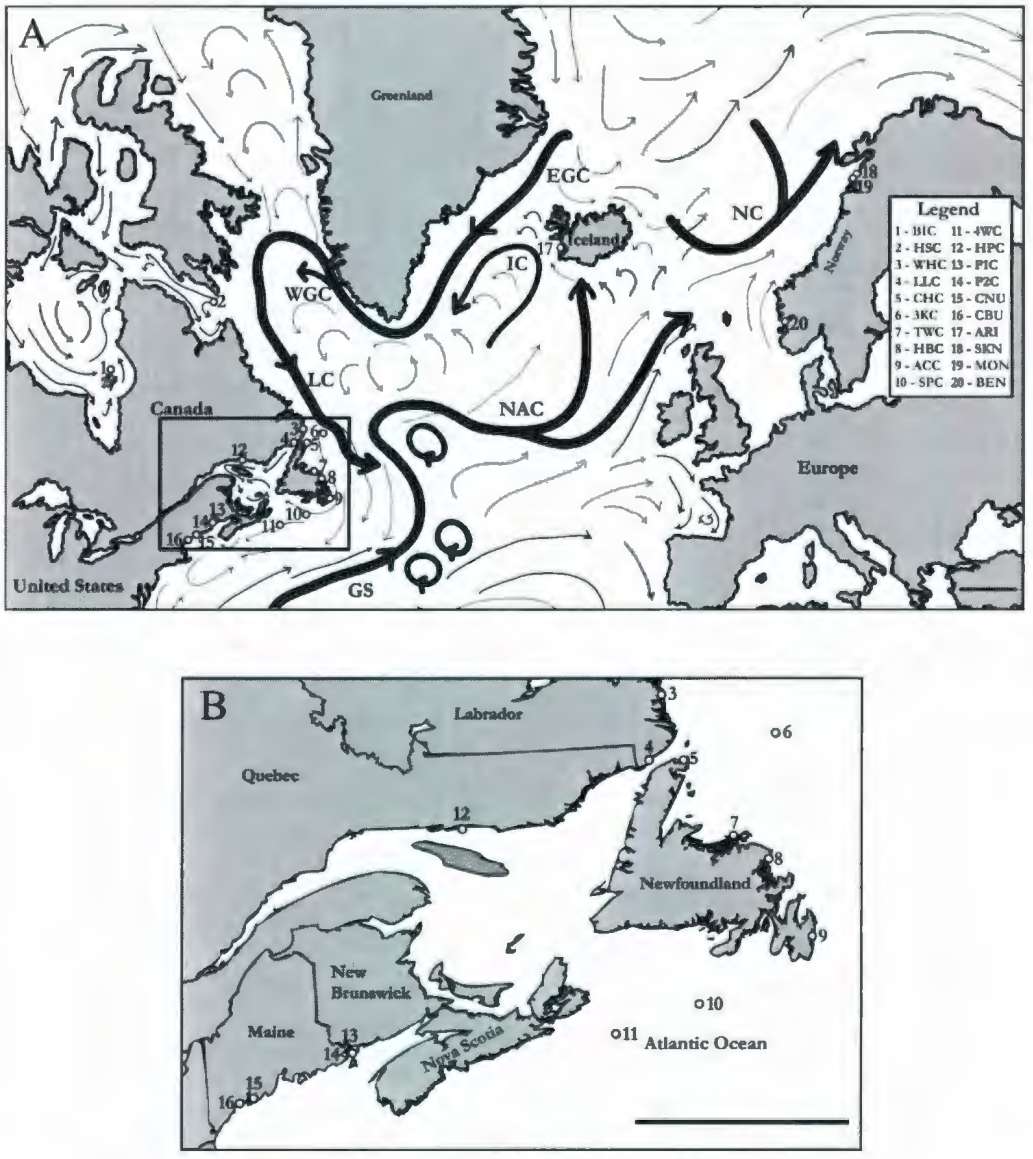

Figure 3-1: A) Schematic current structure of the North Atlantic between April and June (during pelagic larval phase of *Cucumaria frondosa*), adapted from Fratantoni (2001) showing population sites and B) enlarged map of eastern Canada and Maine (USA) from boxed area in A. Scale bar represent 500 km. See Table 3-1 for population identification details. The major currents are labeled as follows; GS-Gulf Stream; NAC-North Atlantic Current; NC-Norwegian Current; EGC-Eastern Greenland Current; IC-Icelandic Current; WGC-Western Greenland Current; LC-Labrador Current. Thickness of arrows indicates strength of current.

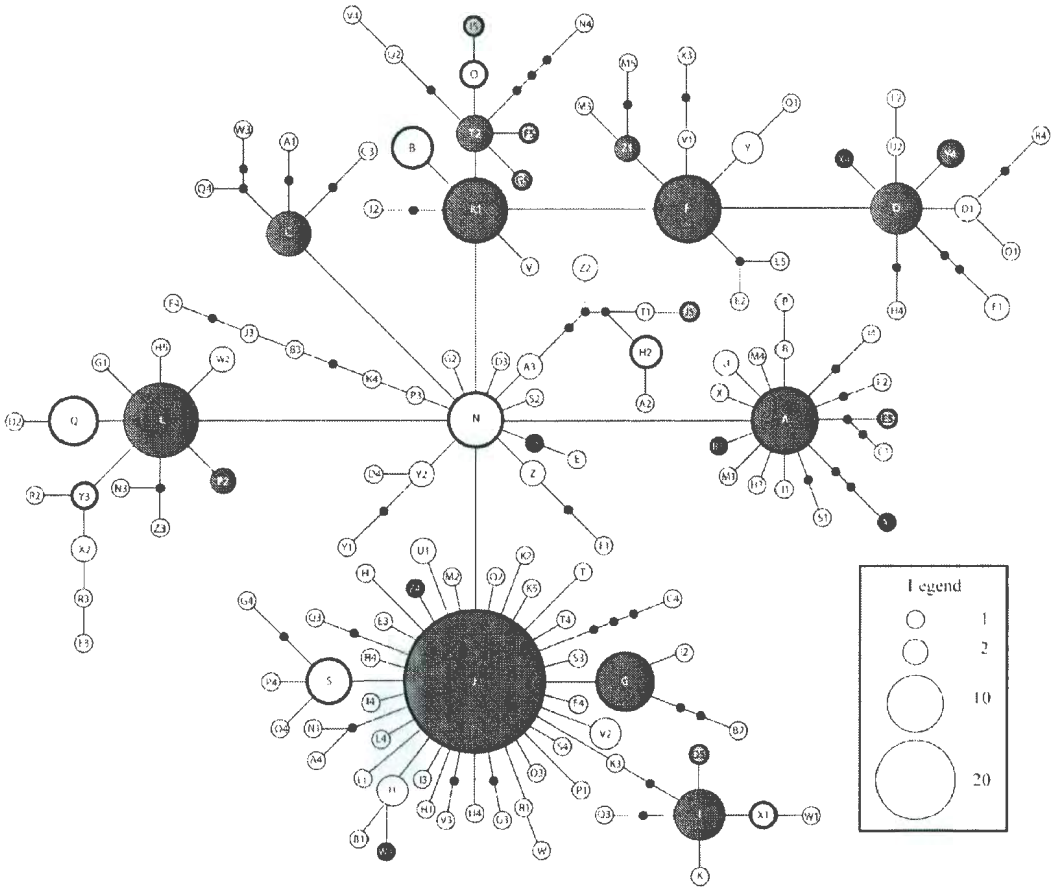

Figure 3-2: Haplotype network of cytochrome oxidase 1 (COI) sequences of *Cucumaria frondosa*. Each haplotype is noted by its corresponding ID. The area of each circle is proportional to the number of individuals with that haplotype. Small black circles represent intermediate haplotypes not found in our samples but needed to link observed haplotypes. Each line in the network represents one nucleotide substitution. Haplotypes from the western Atlantic are shown in white, those from the mid-Atlantic are shown in light grey with a thick black border and those from eastern Atlantic in black. Shared haplotypes between the eastern and western Atlantic are shown in dark grey, between the western and mid-Atlantic are shown in white with a thick black border. Haplotypes shared between all regions are shown in dark grey with a thick black border.

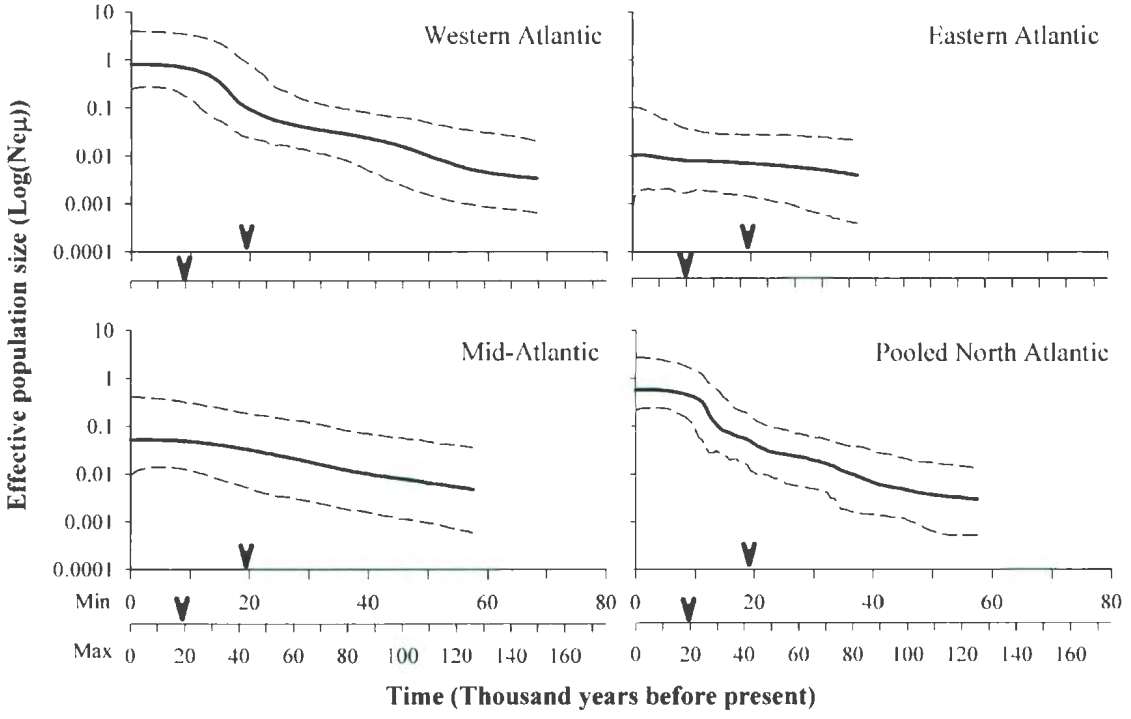

Figure 3-3: Bayesian skyline plots depicting the demographic history of C. frondosa across the North Atlantic. The solid line represents the median value for the log of effective population size (Log N_e) and the dashed lines represent the upper and lower 95 % credible intervals. The x-axes represents thousands of years before 2008 with minimum and maximum time estimates based on previously calculated sea urchin COI mutation rates of 1.6-3.5 % Myr^{-1} (Lessios et al. 1999, 2001; McCartney et al. 2000). Time estimates of the LGM are noted with arrows on the x-axes.

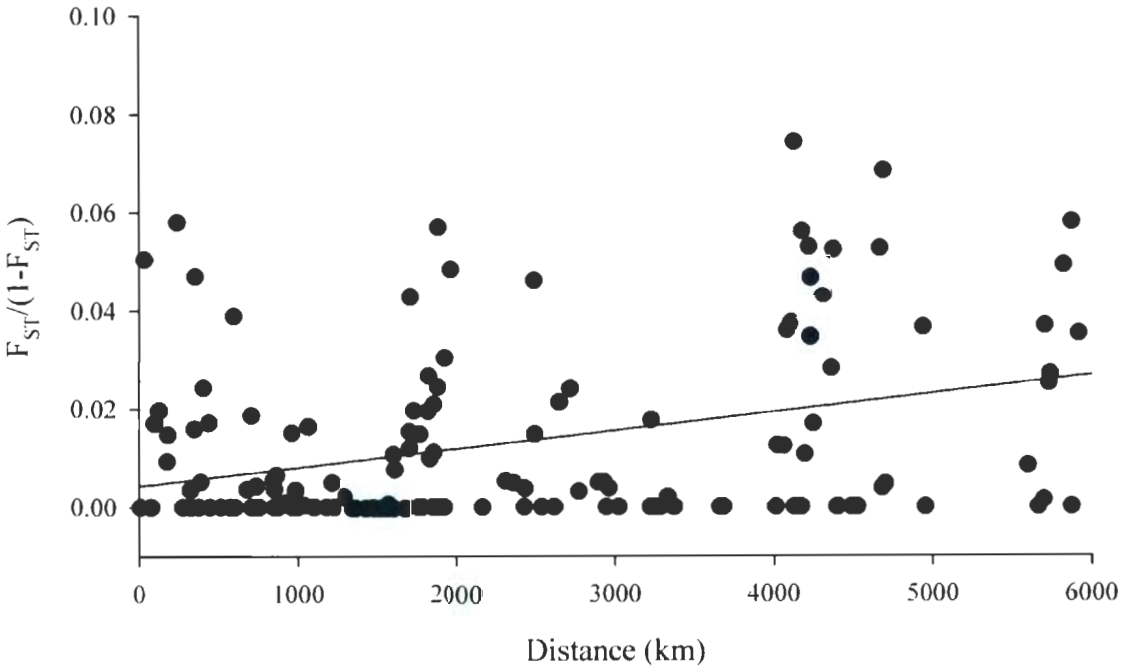

Figure 3-4: Slatkin's (1993) linearized genetic distance ($F_{ST}/(1-F_{ST})$) plotted against geographical distance between samples of *Cucumaria frondosa*. All negative values were zeroed. Mantel's test, r=0.32, p=0.028.

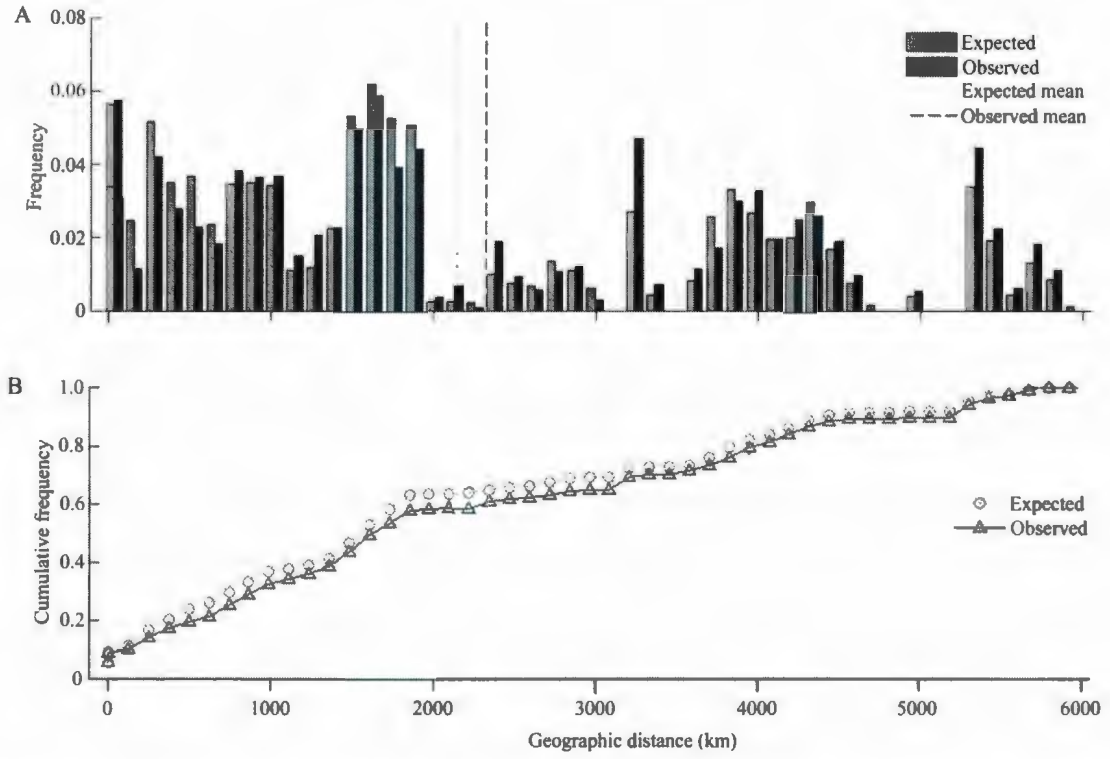

Figure 3-5: A) Distance distribution of expected and observed shared COI haplotypes of *Cucumaria frondosa*. Observed mean=2310.67 km. Expected mean=2130.00 km, p=0.534 B) Cumulative frequency distribution of observed and expected shared COI haplotypes of *Cucumaria frondosa*. D_{cdf}=0.047, p=0.621.

CHAPTER 4: SUMMARY

Cucumaria frondosa *with tentacles extended*

4.1 Biology and ecology of *Cucumaria frondosa*

This study adds key knowledge on various aspects of the biology of *C. frondosa* (Table 4-1) relavant from both ecological and resource management perspectives on a global scale and for the local fishery (Table 4-2). It also provides tools for further studying this and other species of sea cucumbers.

Cucumaria frondosa exhibits a patchy distribution at a mean density of 0.29 ind m^{-2} that varies with substrate type along the St. Pierre Bank (southwest Newfoundland). This species shows selectivity for hard substrata in rocky and gravelly environments that provide refuges for recruits and a hard surface for attachment, necessary for feeding and spawning. Increasing size of sea cucumber was linked with increasing hardness of substrate. Sea cucumbers were also found on other less optimal substrates such as sand where smaller and strangely behaving individuals were found, suggesting that these individuals may not occur there naturally, but as a result of being displaced by storms or currents. Artificially high gonadal index in sea cucumbers from sand substrate was likely the result of reabsorption of the body wall, an energy storage tissue for maintaining reproduction and body function during non-feeding periods. Conversely, a higher body wall thickness was observed in sea cucumbers from soft sand substrate compared to hard mixed gravel and rock substrate and may be the result of individuals rolling along the sandy bottom.

The spawning period for *C. frondosa* in the Newfoundland region occurs from late March to early May in response to increasing phytoplankton abundance. Small sizes of oocytes result in faster development than in other parts of its distribution, leading to

increased local recruitment and small developing larvae. After two years of growth, juvenile sea cucumbers reached a maximum of 6 mm in our study, highlighting the relationship between growth rates and environmental conditions and availability of food.

Immersed weight was the most accurate measurement for determining sea cucumber size without sacrificing the animal; it exhibited a strong relationship with dried weight (r^2=0.97). This method which weighs the sea cucumber while suspended in water negates the effects of retained fluids and would be ideal for growth studies on any species of sea cucumber. Wet weight was also a good measure of size, but showed higher variability. Contracted length and width, either individually or combined (size index), were not well correlated with dry weight. Accurate methods for assessing sea cucumber size are important in the development and management of sea cucumber fisheries.

Peak growth occurred in correlation with spring phytoplankton production in almost all size classes of juvenile and adult sea cucumbers. Null annual growth occurred in several size classes and may be attributed to density in tanks or competition, however it was likely the product of low food levels. Algae-supplemented size classes showed fewer changes in overall size, indicating that more abundant food enabled sea cucumbers to maintain their body size, but that concentrations were not high enough to increase growth rates. Shrinking body size in response to environmental conditions is not uncommon in echindoderms and further complicates sea cucumber growth studies by confounding the relationship between age and size and increasing the difficulty in establishing size classes to estimate growth. In the Newfoundland and Labrador region, estimated growth rates based on accurate immersed weight studies and size-at-age data for 2-y old juveniles

indicate a minimum of 25 years to reach adult size, however higher growth rates may occur in the field.

The sea star *Solaster endeca* is the most important known predator of *C. frondosa* and occurs in areas with high sea cucumber density. Medium-sized sea stars (R: 7-11 cm) dominate the population on the St. Pierre Bank, suggesting higher predation pressure on small-sized sea cucumbers (IW: <2 g). This study revealed that very small *S. endeca* (ca. 2 mm) prey on post-metamorphic juveniles of *C. frondosa* which already express the escape response typical of adults. Prey-predator interactions in very small juveniles of *S. endeca* and *C. frondosa* suggest that these two species have co-evolved, promoting development of these mechanisms from an early age. Predation by *S. endeca* on adult small-sized (IW: 0.6 ± 0.3 g) *C. frondosa* resulted in shorter feeding bouts, there was no statistically significant preference for small sea cucumbers. *Solaster endeca* feeding rates ranged from 2-4 sea cucumbers per month, but ceased in response to temperatures > 14 °C. Feeding bouts (length of time spent eating) was long (ca. 9-13 days) due to the large size of prey and toughness of the body wall. Lower frequency attacks by *S. endeca* were observed at high temperatures (ca. 15 °C) compared to lower temperatures (ca. 6 °C) indicating decreased predation pressure during peak ocean temperatures (10-15°C) from August to October. An extrapolation of these factors indicate that 2 % of sea cucumbers could be removed annually due to predation by *S. endeca*, however this rate assumes that the sea stars feed only on sea cucumbers, and does not include predation on sea cucumbers by fish and other invertebrates. Furthermore feeding rates may be continuous on the St. Pierre Bank where sea cucumbers are fished because bottom temperatures do not exceed 6 °C. *Solaster endeca* selectively preys upon sea cucumbers that exhibit

injuries ressembling those that may be inflicted by benthic trawls. Chemodetection of damaged sea cucumbers will therefore likely attract *S. endeca* to harvested areas. No feeding was observed on damaged or intact *C. frondosa* by the scavenging sea star *Asterias rubens*, however other invertebrate and vertebrate scavengers may feed on sea cucumber remains.

In predation experiments *S. endeca* employed direct and indirect attacks where the sea star would either move straight to its prey, or take a circuitous route. Direct attacks were only observed if *S. endeca* was in close proximity to its prey. Indirect attacks occurred more often with the sea star positioned above its prey enabling it to use its full body weight to capture the sea cucumber. Indirect attacks also allow the predator to remove itself from direct water flow and attack from "down-wind".

The gene flow study indicated that *C. frondosa* populations across the North Atlantic exhibit high diversity within populations, but low diversity between populations. At local levels, genetic patchiness was detected between some populations as a result of adult genetic composition, source of recruitment and post-settlement selection. Gene flow between populations is sporadic and local recruitment is likely more frequent. Small genetic differences were detected between eastern and western Atlantic groups may be due to variable gene flow rates. Gene flow patterns are linked to larval dispersal through ocean currents with high effective migration rates across the North Atlantic. The pattern of genetic diversity of *C. frondosa* populations across the Atlantic indicate that the species persisted through glacial conditions during the Last Glacial Maximum (ca. 18,000 years ago). Apart from larval dispersal, the glacial persistence is likely due to their adaptation to arctic conditions and ecological plasticity. Weak population structure

indicates that low levels of distant gene flow are able to outweigh microevolutionary processes such as genetic drift or mutation.

4.2 Implications for the fishery

Populations of *C. frondosa* in the western North Atlantic are virtually untouched by directed harvests and represent a practically virgin biomass. Annual spawning and long larval development times enable recruitment to distant populations, however the magnitude and consistency of recruitment between populations is likely low. *Cucumaria frondosa* growth rates around Newfoudnland and Labrador are slow due to limited planktonic food availability; therefore heavily fished populations will likely require a long time to recover. The fittest sea cucumbers (those that will survive and reproduce) reside on hard rocky substrate and are reproductively the most important for contributing to recruitment. Using a trawl to fish sea cucumbers may unintentionally help preserve reproductive adults and sources of recruitment as rocky areas with boulders and outcrops result in damaged gear and poor catches. Deep-water populations of *C. frondosa* beyond the photic zone may support recruitment to coastal areas, however their gametogenesis is triggered by cues from shallower populations. These links between populations suggests that intense coastal harvesting may cause cascading negative effects on annual recruitment. These factors indicate that *C. frondosa* is susceptible to Allee effects, where decreasing densities of sexually mature adults cause a proportionate decline in the number of juveniles produced.

Predation by juveniles of the sea star *S. endeca* highlights the negative pressures affecting post-metamorphic juveniles and thus recruitment. Furthermore, adults of *S. endeca* are drawn to damaged sea cucumbers and predation pressure will increase in areas trawled for benthic invertebrates. An increased number of damaged sea cucumbers may also draw predatory and scavenging invertebrates and fish, further affecting sea cucumber populations.

Most of the *C. frondosa* collected in the North Atlantic appear to be part of a large population connected through high gene flow facilitated by long larval life and ocean currents over long ecological time scales (decades or more). Local genetic patchiness can occur, however, with variations in sources of recruitment and dispersal. The presence of genetic patchiness highlights the need for 'local' management through closure of apparently less connected areas.

This project is a major step in creating a successful management plan for the fishery which ensures economic viability and environmental sustainability provided that fisheries managers take a precautionary approach, heed the lessons from overexploited sea cucumber fisheries elsewhere and continue to have the support and co-operation of harvesters, processors and research scientists.

4.3 Future research

Although *C. frondosa* is fairly well known, various aspects of its biology could be investigated to further our general knowledge of this and other species of sea cucumbers.

Additional research would also help refine management goals and practices for the emerging fishery.

- Sea cucumbers are hard to tag due to their lack of hard structures. Previous studies have failed to find a reliable tag that will not be expelled from the body of the sea cucumber. Development of reliable tagging methods for sea cucumbers would be useful for conducting growth studies in the field.
- Field studies of *C. frondosa* could be conducted following individuals using DNA fingerprinting.
- Biochemical analysis could further elucidate differences in potential fitness between sea cucumbers residing on different substrates
- Other gene regions and markers should be used to refine our estimates of population genetic history including microsatellites, allozyme markers.
- Analysis of oocytes of *C. frondosa* from different sites using size and lipid composition may help determine potential distance travelled before settling. The impact of environmental conditions on oocyte size may be elucidated as well.
- Feeding observations of *S. endeca* should be conducted in the field to determine natural feeding cycles and if prey switching between sea cucumber and other echinoderm species occurs. Abundance of *S. endeca* should also be compared between pristine, untouched areas and fished areas to determine if trawling increases predation pressure as predicted. Field studies in fished areas can also indicate whether scavengers prey upon sea cucumber remains.

- Further studies should be conducted on the anti-predatory behaviour of *C. frondosa* such as the ratio between normal size and increased ("bloated") anti-predatory size. The increased size needed for *C. frondosa* to successfully escape an attack from *S. endeca* should also be determined.
- *Solaster endeca* should be observed to determine aggregative feeding behaviour and their effect on predation rates. Several small to medium-sized sea stars may be able to overcome a large sea cucumber's anti-predatory tactics.
- Chemodetection studies should be conducted on *S. endeca* using intact and damaged sea cucumber prey. Although *S. endeca* is known to be able to detect prey, it is not known at what distance it is most effective.
- The species *C. japonica* is almost morphologically identical to *C. frondosa* and has a similar Arctic distribution. Genetic tests may determine whether the species are the result of speciation during glacial periods. Analysis of ossicles in their tissues may also provide key taxonomic information.
- The use of Western blots can determine the presence of light sensitive rhodopsin on various tissues of *C. frondosa* and may show which parts of the sea cucumber are used to detect light.
- Aggregation patterns should be determined for *C. frondosa* to determine if they aggregate according to size or in reference to food and water flow.
- Immersed weight should be correlated with weight of beche-de-mer product. Harvesters would have a more accurate method of determining how much product can be made from fresh catches.

- Size-at-age needs to be investigated for *C. frondosa* in the field as it is necessary for planning sustainable fisheries practices; however no method has been developed yet to determine this.

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4.5 Tables

Table 4-1: Summary of ecological and biological characteristics of *Cucumaria frondosa*

Traits	Characteristics	Sources
Feeding	Feeds on phytoplankton, phytodetritus and other particulate matter.	Hamel and Mercier 1998; This study
	Seasonal feeding attuned to food abundance; can withstand prolonged fasting periods with no detriment to reproduction.	Hamel and Mercier 1996a; 1998; Singh et al. 1998; 2001
	Non-feeding periods result in a shrinking of body size.	David and MacDonald 2002; This study
Reproduction	Iteroparous.	Hamel and Mercier 1995; 1996b; This study
	Size at sexual maturity: 8-11 cm, contracted length.	Hamel and Mercier 1996b; Grant 2006
	Massive annual broadcast spawning correlated with environmental factors.	Hamel and Mercier 1995; 1996b; Singh et al. 2001, This study
	High natural fertilization rates.	Hamel and Mercier 1996c
	Gametogenic synchrony between distant populations by chemical communication.	Hamel and Mercier 1996a; 1999
	Intermediate fecundity (ca. 9,000 eggs).	Hamel and Mercier 1996b, 2008
Larval development and ecology	Naturally long larval development times (ca. 4-7 weeks).	Hamel and Mercier 1996b; This study
	Environmentally dependent larval development rates.	Hamel and Mercier 1996b; This study
	Positively buoyant larvae found high in water column.	Medeiros-Bergen et al. 1995; Hamel and Mercier 1996b; 1996d
	Settle on hard substrates in cryptic habitats.	Hamel and Mercier 1996b; Medeiros-Bergen et al. 1997
Growth	Varies with depth, food availability and size class.	Hamel and Mercier 1996b; This study
	From larvae in the lab: 2-16 mm y^{-1}.	
	From 35 mm in the field: 3-24 mm y^{-1}.	
	Various size-classes in the lab: 0-3 mm y^{-1} (annual growth can be null or weakly negative).	
	All values = contracted length (CL).	
Population ecology	Distributed from shallows to great depths (ca. 20-1300 m).	Jordan 1972; Brinkhurst et al. 1975; Hamel and Mercier 1996a; 2008; J.-F. Hamel personal observation
	Sex ratio of 1:1.	Coady 1973; Hamel and Mercier 1995, 1996b, 2008
	Can colonize bedrock, boulder, gravely and sandy habitats.	Klugh 1923; Hamel and Mercier 1996b; Grant 2006; Grant et al. 2006; CSAR 2006; Kirshenbaum 2006; This study

Table 4-1 continued

Traits	Characteristics	Sources
Population ecology	Can be found in areas of intermediate salinity (e.g. estuaries).	Hamel and Mercier 1996a; Singh et al. 2001
	Adapted to Arctic conditions.	Smirnov 1994; Gudimova et al. 2004
Predation	Well developed anti-predatory response to a predator (*S. endeca*), from post-metamorphic juvenile to adult stages.	Legult and Himmelman 1993; This study
	Damaged sea cucumbers can attract predators and are vulnerable to scavengers.	Robinson et al. 2001; This study
	Juveniles are subject to grazing predation by *Strongylocentrotus droebachiensis.*	Hamel and Mercier 1996b, 2008
Genetic structure	High historical gene flow across the the North Atlantic.	This study
	Genetic patchiness at local levels.	This study
	High genetic diversity as a result of old populations.	This study

Table 4-2: Summary of ecological and biological characteristics of *Cucumaria frondosa* on the St. Pierre Bank.

Traits	Characteristics	Sources
Morphometrics	Dry and immersed weights are the most accurate measures of sea cucumber size.	This study
	Morphometric variations in relation to environment (e.g. smaller individuals found on soft sandy substrate).	This study
Reproduction	Annual spawning from March to May.	Grant et al. 2006; This study
	Size at sexual maturity ca. 9-11 cm contracted length, ca. 7.3 size index.	Grant et al. 2006
Growth	Maximum growth (ca. 1.4 cm mo^{-1}, 0.75 g mo^{-1}, 12-25 % size increase) during periods of high food abundance (spring).	This study
	Various size-classes in the lab: annual [Dec-Dec] growth can be null or very low (0-1.4 cm yr^{-1}, 0.3-1.6 g yr^{-1}).	
	All values = contracted length and immersed weight.	
	Slow growth to commercial size (CL: 15cm) (estimated at min ca. 25 years).	This study
Population ecology	Distributed from 10 to 101 m.	DFO data
	Sex ratio of 1:1.	Grant et al. 2006
	Can colonize bedrock, boulder, gravely and sandy habitats.	Grant et al. 2006; CSAR 2006; This study
	Seasonal variations in body wall thickness.	Grant et al. 2006
Predation	Average size of sea star predator *Solaster endeca* ca. 7.5 cm radius.	DFO data
	Damaged sea cucumbers can attract predators	This study
	Predation rates of adult *Solaster endeca* on adult *C. frondosa* (2-4 sea cucumbers mo^{-1}, 0.012 ind m^{-2} removed annually).	
	Juveniles are subject to predation by juveniles of *Solaster endeca*.	This study
Genetic structure	High west to east gene flow in the North Atlantic.	This study
	Genetic patchiness at local levels.	This study
	High genetic diversity as a result of old populations.	This study

APPENDICES

Appendix 1: List of presentations delivered

1. Science before the fishery – Growth rates, predation, gene flow and substrate related health status of the sea cucumber *Cucumaria frondosa*, Newfoundland and Labrador. Oral presentation at the Sea Cucumber Industry Meeting, May 14-15, 2007. Marystown, NL, Canada.

2. Growth rate and predation pressure as key factors in the management of *Cucumaria frondosa* (Holothuroidea) in Eastern Canada. Poster presentation at the Advancing the Science of Limnology and Oceanography (ASLO) conference, June 8-13, 2008. St. John's, NL, Canada.

3. Science before the fishery – Growth rates, predation, and gene flow of the sea cucumber *Cucumaria frondosa*. Oral presentation at the Atlantic Sea Cucumber Workshop, June 17-18, 2008. Halifax, NS, Canada.

4. Growth rate and predation pressure as key factors in the management of *Cucumaria frondosa* (Holothuroidea) in Eastern Canada. Poster presentation at the 5th North American Echinoderm Conference, July 20-26, 2008. Melbourne, FL, USA.

5. Mitochondrial DNA gene flow analysis in North Atlantic populations of the sea cucumber *Cucumaria frondosa* (Holothuroidea: Echinodermata). Oral presentation at the 5th North American Echinoderm Conference, July 20-26, 2008. Melbourne, FL, USA.

6. Glacial persistence in North Atlantic populations of *Cucumaria frondosa* (Holothuroidea: Echinodermata). Poster presentation at the 13th International Echinoderm Conference, January 5-9, 2009. Hobart, TAS, Australia.

7. Assessment of the biology, ecology and gene flow of the sea cucumber *Cucumaria frondosa* for management of the fishery. Oral presentation at the Sea cucumber Regional Advisory Process, April 6-7, 2009. St. John's, NL, Canada.

Appendix 2: *Cucumaria frondosa* CO1 haplotypes and their frequencies across 20 sample sites in the North Atlantic. See Table 3-1 for sample site identification (ID).

ID	BIC	HSC	CHC	WHC	LLC	CBU	CNU	P1C	P2C	3KC	ACC	HBC	SPC	TWC	4WC	HPC	MON	SKN	BEN	AKI
A	1	1		2		1	1	2				1		1			1			2
B	1		1	2																1
C	1	1									2				1		2			
D	1	1	1				1	1										3		
E	1																			
F	1				1		1	1	2		1	1					3			2
G	1					1		1	1	1				1				3		1
H	1																			
I	1			2													3	2		
J	2	5	4		4	3	3	4	8	1	2	2	3	3	4	2	2	3		6
K	1																			
L	1		2	1	1	2	1				1	1	1		1	2	2	1		
M	1																			
N	1		1	1		1					1		2							2
O		1																		1
P		1																		
Q		1	1		1	1	1	1												1
R		1																		
S		1	2		1											1				1
T			1																	
U			2																	
V			1																	
W			1																	
X			1																	
Y			3																	
Z			1				1													
A1			1																	
B1			1																	
C1			1																	
D1			1				1													
E1			1												1					
F1			1																	
G1			1																	
H1				1																
I1				1																

Appendix 2 continued

ID	BIC	HSC	CHC	WHC	LLC	CBU	CNU	P1C	P2C	3KC	ACC	HBC	SPC	TWC	4WC	HPC	MON	SKN	BEN	AKI
J1				1	1							1								
K1				2			2	1	1	1			1				2			2
L1				1																
M1				1																
N1				1																
O1				1																
P1					1															
Q1					1															
R1					1															
S1					1															
T1					1															
U1					1		1													
V1					1															
W1					1															
X1					1															1
Y1						1														
Z1						1											1			
A2						1														
B2						1														
C2						1														
D2						1														
E2							1													
F2							1													
G2							1													
H2							1													2
I2							1													
J2							1													
K2								1												
L2								1												
M2								1												
N2								1												
T2								1									3			
U2									1			1								
V2									1			1		1						
W2									1			1								
X2									1											

Appendix 2 continued

ID	BIC	HSC	CHC	WHC	LLC	CBU	CNU	P1C	P2C	3KC	ACC	HBC	SPC	TWC	4WC	HPC	MON	SKN	BEN	AKI
Y2									2											
Z2									1					1						
A3									1											
B3									1											
C3									1											
D3									1											
E3									1											
F3									1											
G3										1										
H3											1									
I3											1									
J3											1									
K3											1									
L3											1									
M3											1									
N3											1									
O3											1									
P3												1								
Q3												1								
R3												1								
S3												1								
T3												1								
U3												1								
V3												1								
W3												1								
X3												1								
Y3												1								1
Z3												1								
A4												1								
B4												1								
C4												1								
D4													1							
E4													1							
F4														1						
G4														1						
H4														1						
I4														1						

Appendix 2 continued

ID	BIC	HSC	CHC	WHC	LLC	CBU	CNU	P1C	P2C	3KC	ACC	HBC	SPC	TWC	4WC	HPC	MON	SKN	BEN	AKI
J4														1						
K4														1						
L4														1						
M4														1						
N4														1						
O4														1						
P4															1					
Q4															1					
R4															1					
S4															1					
T4															1					
U4															1					
V4															1					
W4																	1			
X4																	1			
Y4																	1			1
Z4																			1	
A5																			1	
B5																			1	
C5																			1	
D5																				1
E5																				1
F5																				1
G5																				1
H5																				1
I5																				1
J5																				1
K5																1				
L5																1				
M5																1				